FACTORS THAT INFLUENCE THE FUNCTION OF CARDIAC ADRENOCEPTORS

by

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I HEREBY DECLARE THAT THIS THESIS IS COMPOSED ENTIRELY BY MYSELF AND THAT THE WORK IS MY OWN; OTHER PEOPLE'S CONTRIBUTIONS HAVE BEEN ACKNOWLEDGED WHERE APPROPRIATE.

ABSTRACT

In view of recent reports that both β_1- and β_2- adrenoceptors may co-exist in the same organ and subserve responses in the same direction, we set out to investigate factors influencing their relative densities and responses to adrenergic stimuli. We have studied the effects of sympathectomy on cardiac β-adrenoceptors of the guinea-pig heart and found both innervated and discrete sub-populations. While full adrenergic agonists appear to exert their chrono- and inotropic effects via the β_1-adrenoceptor, salbutamol (previously termed a partial agonist) appears to have the dual action of a prejunctional β_2 agonism, hence releasing endogenous transmitters, and a postjunctional β_1 antagonism, thereby reducing the effects of full adrenergic agonists in 6-hydroxydopamine - denervated atria. 6-OHDA pretreatmant was shown to double guinea-pig cardiac binding sites for the radioligand (-)^{125}I-pindolol with no alteration in affinity. There was an increased β_1 : β_2 ratio in both atria and ventricles. The resutls produced by radioligand binding studies do not appear to be directly compatible with the functional adrenoceptor and may therefore only be termed 'binding sites'.
In the light of conflicting reports on the effects of thyroid hormones on the responsiveness of the heart to adrenergic stimuli, we investigated alterations in canine ECG parameters by thyroid hormones in terms of

effects of β-blockers. Sotalol, but not propranolol, was found to decrease the heart rate in the tachycardia produced by T_3 administration. This suggests a direct thyroid hormone action on the canine myocardium rather than an enhanced sensitivity to catecholamines in hyperthyroidism. β-blocker toxicity studies yielded the information that neither sotalol nor nadolol abolished sinus rhythm up to the time of death whereas propranolol produced a 2 : 1 atrio-ventricular block. Death due to β-blocker overdose appears due to respiratory depressant actions, apparent from the blood pCO_2 and pO_2 levels during β-blocker infusion, and from the fact that higher lethal doses were necessary in the artificially-ventilated animals. These depressant effects are unrelated to the pharmacokinetic properties of the drugs and appear to involve both central and peripheral mechanisms.

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SECTION I : INTRODUCTION AND REVIEW OF THE LITERATURE

The initial fundamental observations on the specificity of adrenergic agonists to evoke responses in various tissues were made as early as 1906 by Dale when he found that the physiological effects of certain ergot alkaloids such as cornutine and sphacelotoxine fell into two distinct groups. The first group of effects were those of smooth muscle stimulation eg. arterial, uterine and iris sphincter contraction while the second group were those of inhibition of adrenaline-stimulated sympathetic innervation. In the cat, the increase in arterial blood pressure in response to adrenaline could be reversed to a decrease by ergot. This 'adrenaline reversal' led much later to the conclusion that there were two receptor pools acting in opposite directions. This hypothesis was proposed by Ahlquist (1948) and replaced the earlier idea that different sympathomimetic amines had either excitatory or inhibitory actions. This was on the basis of a single rank potency order for a series of adrenergic agonists i.e. adrenaline > noradrenaline > methylnoradrenaline > isopropylnoradrenaline for vasoconstriction, gut inhibition and pupil dilatation while an entirely different rank order of potency of isopropylnoradrenaline > adrenaline > methylnoradrenaline > adrenaline existed for myocardial stimulation and vasodilatation. The hypothesis of adrenoceptor subdivision into α- and β-subtypes was

therefore suggested. In 1967, Lands et al investigated these proposed subtypes more deeply. They suggested a further subdivision of the β-adrenoceptor into β_1 and β_2 subtypes. Again this was done on the basis of two distinct patterns of tissue responses emerging to a series of adrenergic agonists. They found that adrenaline $\geq$ noradrenaline for myocardial stimulation and lipolysis while adrenaline >> noradrenaline for bronchial relaxation and vasodilatation. They postulated that the former was mediated via β_1-adrenoceptors and the latter via β_2-adrenoceptors. They however noted that the 'preference' of the β_1- and β_2-adrenoceptor agonists and antagonists was not absolute and that multiple effects may therefore be observed. On this basis, however, it became accepted that the β-adrenoceptors of the myocardium were of the β_1 subtype and drugs acting on β_1 receptors, and consequently the heart, became termed 'cardioselective' while those adrenoceptors of the airways were of the β_2 subtype and drugs acting predominantly on β_2 adrenoceptors were termed 'bronchioselective'. Black (personal communication) and Ungar (1979) suggested a further modification of the system that all β_1-adrenoceptors are innervated whereas β_2-adrenoceptors may be widely distributed and more responsive to circulating catecholamines. In 1971, Sutherland put forward the concept of the existence of a 'second messenger' which associates the adrenergic response with an alteration in the cellular adenylate cyclase concentration. It

subsequently became accepted that β_1- and β_2-adrenoceptor activation is very often associated with adenylate cyclase activity with a subsequent rise in intracellular cyclic AMP concentrations whereas some α-adrenoceptor mediated responses such as those of the human platelet are associated with a depressed adenylate cyclase activity with a resultant fall in cyclic AMP levels. Indeed, it is often now stated that the efficacy of a β-adrenoceptor agonist may be regarded as its ability to activate the cellular adenylate cyclase system (Kent et al, 1979). This adrenoceptor subdivision was investigated by Carlsson (1972,1977). He found that selective antagonists inhibited agonist actions in different orders in the cat heart. He found that metoprolol (β_1-selective) antagonised noradrenaline > adrenaline while butoxamine (β_2-selective) antagonised adrenaline > noradrenaline; practolol (β_1-selective) antagonised the responses to isoprenaline, adrenaline, noradrenaline and salbutamol to different extents with a different order being produced by β_2-selective H35/25. His results were best fitted by the hypothesis that both β_1 and β_2 subtypes of adrenoceptor coexist in some tissues such as the cat heart and that responses to adrenaline and noradrenaline are at least partly mediated via different β-adrenoceptors. A further complication of the adrenoceptor population was suggested by Govier (1968) in guinea-pig right atria and by Kaumann (1977) in kitten papillary muscle that when more than 90% of

the β-adrenoceptors are occupied by a specific antagonist, a small component of the remaining positive inotropic effects of noradrenaline (but not isoprenaline) may be antagonised by α-adrenoceptor antagonists. This indicates multiple contributions of several receptor subtypes to the same response. It was therefore of great interest to us to investigate the relative numbers of cardiac β_1 and β_2 receptors and whether the subpopulations are influenced by factors of innervation i.e. are discrete β-adrenoceptors unaffected by alterations in sympathetic supply to a tissue whereas innervated receptors are greatly affected or are the two independent of tissue innervation?

Often the best way to investigate the physiological and pharmacological contributions of a system is to 'remove' it and observe the results. 'Removal' of the sympathetic nervous system by various means is well-documented as resulting in a supersensitivity of the organ concerned to catecholamines. Trendelenberg and Weiner (1962) described two mechanisms whereby supersensitivity may develop. The first, a presynaptic supersensitivity, is seen following the application of cocaine and desipramine or 24 hours following surgical denervation and they attributed this to an impairment of noradrenaline uptake into the presynaptic nerve terminal leaving a larger concentration free to evoke a response. The second, a postsynaptic supersensitivity, is due to an increased β-adrenoceptor density which occurs 12 - 16 days after surgical sympathectomy. A denervation supersensitivity

following degeneration of the sympathetic nervous system as a result of multiple system atrophy (Shy-Drager syndrome) is well-documented by Bannister et al (1979, 1981). They found that β-adrenoceptor density was greatly increased in lymphocytes in the syndrome with no alteration of receptor affinity. If lymphocyte receptor density can be regarded as reflecting the state of the cardiac adrenoceptors, then this increased receptor density may account for the increased sensitivity to isoprenaline observed in the syndrome.

Supersensitivity to catecholamines is widely-reported. Banerjee et al (1977) demonstrated an increased β-adrenoceptor concentration in rat skeletal muscle following surgical denervation. Broadley and Lumley (1977) found that reserpine pretreatment shifted both the rate and tension log dose - response curves of isolated guinea-pig atria to isoprenaline to the left. Since no alteration in tissue response to Ca^{2+} ions was demonstrated, it indicated that the resultant supersensitivity was specific for β-adrenoceptors. They also found that salbutamol (which produced only a sub-maximal response in control atria which they termed partial agonism) also evoked responses at a lower concentration.

Another method of sympathectomy - by 6-hydroxydopamine (6-OHDA) - has proved popular. Following the suggestion of Senoh et al (1959) that 6-OHDA may be formed by dopamine auto-oxidation, further pharmacological investigations revealed that it could be employed to

produce a long-term efficient depletion of peripheral sympathetically-innervated organ catecholamines (Porter et al, 1963). Mueller et al (1969) reported that 6-OHDA produced a selective destruction of postganglionic adrenergic nerve terminals and a long-term depletion of noradrenaline in sympathetically-innervated organs. In 1974, Kostrzewa and Jacobowitz showed that 6-OHDA was selectively taken up by the peripheral nervous system into noradrenergic neurones. This neuronal accumulation of 6-OHDA appears to be prerequisite for its destructive effects (Stone et al, 1964). Since it was demonstrated that the accumulation of ^{3}H-6-OHDA by mouse atria *in vitro* that had previously been surgically sympathectomised was significantly depressed, the actual amount taken up being identical to that in the presence of desipramine, and since desipramine inactivates the monoamine uptake mechanism (Axelrod et al, 1961) it was proposed that in order to effect a degeneration of the sympathetic nervous system, 6-OHDA is first taken up into noradrenergic neurones by the active amine pump. This was supported by Stone et al (1964) who found that imipramine (also an uptake blocker) prevented noradrenaline depletion produced by 6-OHDA in mice. After active uptake. 6-OHDA becomes concentrated in the amine storage granules (as much as 50%) with a concomitant decrease in their noradrenaline content. The granular uptake of ^{3}H-6-OHDA was demonstrated in the cat spleen by Thoenen and Tranzer (1963) who showed at low doses that it could

be released as a false transmitter. Electron microscopic studies have revealed that cholinergic neurones, non-adrenergic neurones, myelinated axons, endothelial cells and smooth muscle cells are unaffected by 6-OHDA while the noradrenergic neurone degenerates (Siggins and Bloom, 1970; Tranzer and Thoenen, 1967). Twelve hours to three days following administration of 6-OHDA to cats and rats, the adrenergic nerve terminals were found to be in various stages of degeneration. In many cases, the Schwann cells were seen to engulf the nerve terminals which appeared to lyse and the plasma membrane become indiscernible from that of the Schwann cell (Thoenen, 1972a). The selectivity of neuronal destruction by 6-OHDA is best seen in organs such as the vas deferens where cholinergic and adrenergic nerve terminals lie very close together, often surrounded by the same Schwann cell, yet the cholinergic nerve remains intact. Thoenen and Tranzer (1968) stressed that a critical intraneuronal level of 6-OHDA must be attained to precipitate the degenerative process. This critical level appears to be very high in some cases, Jonsson and Sachs (1971) quoting it as being 1% tissue weight. It is very interesting to note that the cell bodies of adult rats and cats display no changes following 6-OHDA treatment (Thoenen and Tranzer , 1973) even if very high doses are applies topically or by close-arterial infusion. This resistance of the cell body is the probable explanation of the complete recovery and regeneration of the sympathetic nervous system in the adult animal when treatment

is ceased whereas in newborn animals the entire neurone is destroyed and the resultant sympathectomy is permanent (Tranzer et al, 1969). This discrepancy in the severity of the effects of 6-OHDA in adult and newborn animals is thought to be due to a shift in the transport efficiency from the cell body to the periphery during the animal's development into adulthood (Thoenen, 1972b). Heusler (1971) showed that thirty minutes following a toxic dose of 139mg/kg 6-OHDA administered intravenously, the constrictor response of isolated cat mesenteric arteries to noradrenaline was significantly depressed whereas responses to $CaCl_2$ and KCl were normal indicating a specific effect of 6-OHDA and not a non-specific smooth muscle depressant action. If this was repeated following phentolamine pretreatment, the responses were as in controls after twenty hours indicating an α-adrenoceptor mediated effect of 6-OHDA. However, the time lag of twenty to forty-eight hours may indicate that there was covalent bonding of 6-OHDA to the α-adrenoceptors of configurational changes had been produced which discouraged agonist binding. The specificity of 6-OHDA for the sympathetic nervous system made it an ideal tool for the present study to investigate alterations in cardiac β-adrenoceptors following sympathectomy both of a permanent (newborn-treated) and a reversible (adult-treated) nature. We wished to investigate the effects of 6-OHDA-induced denervation on cardiac adrenoceptors. One method of receptor study that has become ever-increasingly employed in recent years is that of the

radioligand binding study. When they were introduced, it was immediately inferred from pharmacological and physiological studies that a true adrenergic receptor should demonstrate certain corroborative features in the binding study to confirm their role *in vivo*:

1) binding should demonstrate stereospecificity and (-) isomers should on the whole be more potent than (+) isomers.
2) the rank order of potency of drug binding in the isolated membranes should reflect that in the intact tissue.
3) binding should be saturable i.e. a finite receptor population should be demonstrable.

In 1972, Lefkowitz and Levey solubilized a fraction of cat myocardium that had adenylate cyclase activity and that would also bind catecholamines in the same order as *in vivo*, and would therefore offer a study model. The production of a suitable radioligand then became the problem. One of the first β-adrenoceptor agents to be used was ^{3}H-propranolol (Levitski et al, 1974) but it had such a low specific activity and produced such high non-specific binding that it gave only very limited information. In 1974, Lefkowitz et al developed the high-affinity ^{3}H-dihydroalprenolol which bound to putative β-adrenoceptors in the frog erythrocyte membrane, a similar class of binding site being identified in turkey erythrocytes by Aurbach et al (1974) using the alternative high affinity label of ^{125}I-hydroxybenzylpindolol.

Since neurotransmitter receptors are thought in most cases to consist of ay least two components - a) the recognition or binding site and b) a 'translating' portion to convert the transmitter recognition into an activation of the second messenger, the effects of adrenergic agonists on the cyclic AMP concentration *in vitro* should mirror the pharmacological effects *in vivo*. Harden et al (1976) showed that the order of potency of an adrenergic agonist series to inhibit ^{125}I-hydroxybenzylpindolol binding and to activate cyclic AMP production were the same and they therefore concluded that the binding sites visualised by radioligand binding may be representative of the physiological receptor. Lefkowitz and Levey (1972) found that adenylate cyclase activity in their cat myocardial preparation was not activated until the addition of phosphatidylinositol. This indicated that the adenylate cyclase system was located internally from the β-adrenoceptor (since it utilised intracellular ATP as a substrate to produce cyclic AMP) requiring the 'lipophilic bridge' of inositol to act as a coupling factor. Multiple criteria therefore appear to be satisfied to equate membrane binding sites with a physiological adrenoceptor and therefore binding studies may be regarded as a useful tool for receptor study provided that certain requirements are met.
Since from the literature it had become apparent that many factors may be responsible for altering the β-adrenoceptor mediated response to stimulation by catecholamines, it would be interesting to investigate a

further machanism whereby β-adrenoceptor mediated responses may be affected - by alteration of thyroid state. As we planned to investigate a series of β-adrenoceptor antagonists throughout the study, observing their effects in changing thyroid state would afford an opportunity to study the antidysrhythmic properties of antagonists from all three classes proposed by Vaughan Williams (1974) :

Class I : these drugs decrease the maximum rate of depolarisation of cardiac muscle but produce no alteration in resting potential. They restrict entry of the depolarising current and therefore repolarisation must proceed further before the ion gates are open sufficiently for the minimum rate of rise to produce contraction propagation. Often termed 'membrane-stabilizing' drugs.

Class II : these drugs are antisympathetic, either by virtue of producing β-blockade or by inhibition of endogenous transmitter release.

Class III : alteration of thyroid state has no effects on action potential voltages but large effects on the duration of action potential. These drugs act to prolong action potential duration and are often termed 'anti-thyroid' because they mimic the effects of hypothyroidism on the myocardium without themselves altering thyroid state or any other manifestations of thyrotoxicosis.

None of these classes of drugs are mutually-exclusive, however, and most drugs overlap in their actions. It is often unquestioningly accepted that thyroid hormones increase the sensitivity of cardiac tissues to β-adrenergic agonists and that this may be corrected by epidural blockade (Leak, 1963) indicating an increased sympathetic activity. Indeed, many of the clinical manifestations of the thyrotoxic state are consistent with a hyperadrenergic state, eg. increased heart rate, O_2 consumption, cardiac output and ventricular stroke work. However, in 1971 Levey searched the existing literature to assess the evidence given for a relationship between hyperthyroid state and the adrenergic system shown by an enhanced cardiac sensitivity to infused catecholamines and by a catecholamine-sensitive adenyl cyclase system, but he failed. It is therefore necessary that a further investigation of the relationship between the adrenergic system and thyroid state be carried out. In 1969, Wiener et al found that reserpine, α-methylDopa and guanethidine all reduced the heart rate and cardiac output in thyrotoxicosis. As left ventricular tension time is often described as the major determinant of myocardial O_2 consumption, the ratio of LV work : LV tension time may be used as an index of myocardial efficiency and this is increased 14% by propranolol in thyrotoxicosis. In 1977, Williams and Lefkowitz demonstrated that T_3 and T_4 treatment of the rat myocardium resulted in more than doubling of the β-adrenoceptor populations with no alteration in receptor

affinity and this was confirmed by Tsai and Chen (1978) and Tse et al (1980). They proposed that this receptor concentration increase accounted, at least in part, for the enhanced responses they observed in hyperthyroidism to catecholamines, and further that the mechanism for receptor induction may be mediated by a T_4-induced increase in RNA synthesis. They also found that hyperthyroid rats produced more cyclic AMP in response to isoprenaline. Their findings were given support by Ciaraldi and Marinetti (1977) who went further to demonstrate a concurrent decrease in α-adrenoceptor density with a decrease in affinity. When they extended the study to include the hypothyroid rat, they found that the β-adrenoceptor population was decreased. This was confirmed in 1978 by Giudicelli who found that adipocytes taken from thyroidectomised rats had only 1/3 of the β-adrenoceptor population of euthyroid rats and that this could be corrected by T_3 therapy. These binding studies consistently suggested that there was a gross alteration at adrenoceptor level, that these receptors were functional (indicated by increased cyclic AMP production) and therefore capable of altering responses to exogenous and endogenous catecholamines. Wildenthal (1974) found an *in vitro* mouse heart culture system where two days exposure to T_3 produced no alteration in rate responses of the atria to theophylline, glucagon or acetylcholine but where responses to submaximal doses of noradrenaline were significantly increased. He concluded from this result that high levels

of T_3 do not produce a hypersensitivity to all chronotropic agents, but only those which produce a chronotropic effect via the β-adrenoceptor. The specificity of action was confirmed by Malbon et al (1978) who found that T_4 treatment produced an increase in β-adrenoceptor mediated cyclic AMP accumulation in fat cells and adipose tissues. Hashimoto and Nakashima (1978) showed that T_4 treatment of guinea-pig atria produced an increase in β-adrenoceptor mediated inotropic responses. The correlation of thyroid and adrenergic states is not always clear-cut, however, McDevitt et al (1978) found no significant alteration in responsiveness to isoprenaline in hyperthyroid dogs becoming euthyroid on T_3 treatment or, indeed, between the two extremes of hypo- and hyperthyroid states. Wilson et al (1966) found that neither oral nor intravenous nethalide (β-antagonist) produced any significant alteration in resting heart rate, blood pressure, cardiac output or O_2 consumption and concluded that the changes in cardiac performance manifest in hyperthyroidism are not mediated via the β-adrenergic system. Taylor (1983) showed that T_4 had no effect on noradrenaline (β_1) or terbutaline (β_2) evoked relaxation of the guinea-pig trachea. It therefore appears from the literature that any alterations in tissue responsiveness to adrenergic agents mediated by changes in thyroid state may be specific for that given tissue rather than for all β-adrenoceptor systems or subtypes. We thus chose to investigate the effects of a set of three β-antagonists in the conscious dog during

alteration of thyroid state either by carbimazole to render them hypothyroid or by T_3 treatment to mimic hyperthyroidism. The drugs chosen for the study were propranolol (Classes I and II antidysrhythmic properties), sotalol (Classes II and III) and nadolol (Class II and possibly III). We were particularly interested in Class III activity since it has been associated with the thyroid state by Vaughan Williams (1974). Sotalol is reported to greatly prolong action potential duration by a slowing of the terminal phase of cardiac muscle relaxation and also by producing a small active tension effect (Kaumann and Olson, 1968). However, the QT interval used often to assess this is not purely indicative of the duration of action potential. The action potential does not follow the same time course throughout and the QT interval is only indicative of action potential duration if all ventricular fibres fire synchronously. Any condition producing asynchronous activation of the ventricular fibres would therefore produce a QT prolongation without necessarily affecting action potential duration. Such conditions as the 'long QT syndrome' are associated with ventricular dysrhythmias in contrast to a prolonged action potential duration which, whether drug- or hypothyroidism-induced, is associated with stable rhythm. It would therefore be of great interest to observe Class III activity in altered thyroid states on the electrophysiological parameters of the dog ECG and to correlate these with reports in the literature. Sotalol is reported to be devoid of Class I activity,

having only 1/300 activity of propranolol as a local anaesthetic (Singh and Vaughan Williams. 1970). This is due to sotalol having the same side-chain as isoprenaline but with an electron-withdrawing group (methyl sulphonamide) in the p-position of the aromatic ring and this reduces local anaesthetic activity and greatly increases β-blocking potency (Singh and Vaughan Williams, 1971). Nadolol, on the other hand, is a relatively new β-blocking drug. It was shown by Vukovich et al (1976) to produce a reduction or remission in subjects with frequent ventricular or supraventricular ectopic beats and other cardiodysrhythmias and since these may be associated with thyroid dysfunction, we thought it a useful drug to include in the study. These three blockers also cover a wide range of pharmacokinetic properties. Propranolol is approximately 90% plasma protein bound whereas sotalol is 54% and nadolol only 25-30% bound. Reports on this do differ though, Patel and Turner (1981) finding sotalol not to be significantly bound at all. The drugs also cover a wide range of lipid solubilities, see Table 10. The study would therefore be more complete if it was to include a toxicity test comparing the different pharmacokinetic properties with the effects on the parameters of the ECG. Sibley et al (1978) carried out an extensive series of β-blocker toxicity tests and found that nadolol was of a very low toxicity and we wished to see if this result was confirmed in our hands. The large range of lipid solubilities of the three drugs afforded us an oppor-

tunity to attempt to separate their central effects from their peripheral actions. Since the blood-brain barrier acts as a lipid membrane (Mayer et al, 1959). lipid soluble propranolol would be expected to penetrate it while sotalol would not, nadolol being an intermediate case. When Mustchin et al (1975) showed that a single oral dose of 80mg propranolol in humans significantly depressed the ventilatory response to CO_2 and also depressed inspiratory pressure, it was taken that propranolol possesses central respiratory depressant effects. We therefore concluded that a model was required to correlate the β-blocker cardiac and central effects.

SECTION II : METHODS

1. Preparation of isolated atria.

Guinea-pigs of either sex in the weight range of 300-500g were killed by a blow to the head and exsanguinated. The heart was excised and the atria separated (Hashimoto and Nakashima, 1978; Hughson and Ledsome, 1975). Right atria were mounted on tissue holders with cotton thread and allowed to run spontaneously while left atria were mounted on a combined tissue holder and pacing electrode and were paced at 210 beats/minute at 2V. Right atria were suspended in a 10ml organ bath and left atria in a 30ml bath. Both were bathed in Krebs' solution of the millimolar concentration : Na^+ 141.0; K^+ 5.9; Ca^{2+} 2.6; Mg^{2+} 1.2; Cl^- 104.8; $H_2PO_4^-$ 2.2; HCO_3^- 24.9; SO_4^- 1.2;glucose 11. The solution was gassed with 5% CO_2 in oxygen and maintained at 37°C in the presence of 1.6×10^{-5}M phenoxybenzamine and 10^{-5}M metanephrine to inhibit α-adrenoceptor stimulation and extraneuronal uptake respectively (Lumley and Broadley, 1977; Kaumann et al, 1978). Isometric contractions were recorded and rate responses were calculated by an analogue-logic computer (EAL 380) and tension responses were recorded on an Electromed devices heat pen recorder.

Preparation of isolated tracheae.

Guinea-pigs of either sex were sacrificed as previously described. Tracheae were excised and cut spirally so as to provide 2 or 3 preparations. Sections

of approximately 2cm length were mounted on tissue holders with cotton threads and suspended in 10ml organ baths in the conditions as described for atria. Tension was allowed to develop spontaneously, no carbachol was used.

Construction of log dose - response curves.

All preparations were allowed to equilibrate for at least 30mins during which time the bathing medium was changed several times. A preliminary dose - response curve to an agonist was constructed until a maximum response was obtained. This first dose - response curve of the experiment served to prime the tissue and was not included in subsequent analysis of data. When rate and tension levels returned to their pre-drug values, further dose - response curves to each agonist in duplicate were obtained. An antagonist was then added to the bath and incubated for 1 hour before reconstructing dose - response curves to each of the agonists. Antagonist equilibrium association constants were calculated from the formula

$$K_a = \frac{DR - 1}{B}$$

where DR represents the resulting dose ratio and B the antagonist concentration.

Histochemical fluorescence of cardiac monoamines.

To visualise the cardiac monoamines of the control and 6-OHDA - denervated guinea-pig, the sucrose - phosphate - glyoxylic acid (SPG) method was used because of its reproducibility of discrete fluorescence production and its rapidity. A fresh SPG solution was prepared prior to beginning each experiment as recommended by de la Torre and Surgeon (1976). The solution was of the following composition : 10.2g sucrose + 4.2g K_2PO_4 dissolved in 100ml distilled water and 1.5g glyoxylic acid crystals

added. pH was adjusted to 7.4 with 2N NaOH and made up to a final volume of 150ml with distilled water.

Tissues were excised, the atria and ventricles were separated and frozen in liquid isopentane cooled with liquid nitrogen (b.p. isopentane 36°C). The tissue to be examined was then mounted onto a cryostat chuck using a few drops of water and frozen to a cryostat temperature of -20°C. 12µm sections were taken and lifted straight onto the slides from the cryostat blade, melting onto the slide by virtue of the temperature difference. Several slides were then dipped 3 times into the SPG solution. Excess solution was removed with filter paper at the edge of the slide and the remainder evaporated with a cool jet of air. The slides were then heated in an oven at 80°C for approximately 5 mins. A few drops of mineral oil at 80°C were then placed on the slide and mounted with coverslips. Slides were then heated for a further 2 mins on a hotplate to remove any air bubbles and to allow the oil to distribute evenly. The slides were then examined under a fluorescence microscope fitted with a mercury lamp, dark field condenser and a cut-off filter at approximately 500nm. Photographs were taken on Ilford FP4 film, ASA 125, developed with Ilford Microphen at 20°C and fixed with Ilford Hypam rapid fixer.

3. Drug Treatments.

Neonatal guinea-pigs were given 50mg/kg 6-OHDA intraperitoneally (IP) on days 1,3,5, and 7 after birth, and

denervation then persisted indefinitely. Adult animals were treated with the same dose IP on days 1,2,9 and 14 and sacrificed on day 16. 6-OHDA was made up in 5% ascorbate. Guinea-pigs were reserpinised by 5mg/kg IP on each day of the 2 weeks preceding sacrifice.

4. Radioenzymatic determination of plasma and tissue catecholamines.

Principle: The catecholamines adrenaline (Ad), noradrenaline (NA), and dopamine (DA) are converted to their respective 3-O-methylated derivatives using catechol-O-methyl transferase (COMT) and the radioactive methyl donor (^{3}H)-methyl-S-adenosylmethionine (SAM). The radioactive products of metanephrine (from Ad), normetanephrine (from NA) and methoxytyramine (from DA) are purified by selective extraction and separated by thin layer chromatography (TLC). Metanephrine and normetanephrine are finally oxidised to vanillin. The method is modified from that of da Prada and Zurcher (1976).

Extraction of catechol-O-methyl transferase.

All procedures were carried out on melting ice or at 4^{o}C. 100g of rat livers obtained from 10 rats were homogenised in 400ml 1.19% KCl and filtered through two layers of nylon gauze. The homogenate was centrifuged for 10 mins at 15000 rpm using a refrigerated centrifuge (MSE 18). The white fluffy floating layer was aspirated and discarded. The supernatant was then spun for 30mins at 65000 rpm. The pH of the supernatant was then adjusted to 5.0 using 1M acetic acid and left standing for 20 mins.

The precipitated proteins were spun down at 24000 rpm and discarded. Finely-powdered ammonium sulphate (1.61g/100ml) was then added very slowly under constant stirring. The precipitated proteins wer spun down after 30 mins at 65000 rpm and discarded. More ammonium sulphate was added to the spernatant (14.8g/100ml) and left to stand for 15 mins. The precipitate from this stage, containing COMT, was packed by centrifugation (10 mins at 15000rpm). The pellet was resuspended in 30 ml 1mM $NaPO_4$ buffer at pH 7.0 and dialysed for 15 hours against 5l 1mM $NaPO_4$ buffer (pH 7.0) containig 77.5mg dithiotreitol (DTT) with 48 mm circumference dialysis tubing cleaned 3 times with 10^{-4}M EGTA. The precipitated proteins were sedimented by 10 mins at 15000 rpm and the clear supernatant aliquoted into 1ml cups and frozen.

Reagents:

1) ^{3}H-methyl-S-adenosylmethionine, Radiochemical Centre, Amersham, specific activity 10Ci/mmole.
2) Hydrochloric acid;HCl Analar, BDH-10125 37% made up with double-distilled water (DDW).
3) Perchloric acid;$HClO_4$, BDH Analar, 72%, d=1.7,in DDW.
4) Acetic acid; glacial, BDH Analar,d=1.05, in DDW.
5) Sodium hydroxide; NaOH, BDH Analar.
6) Ammonia; ammonia p.a., Merck 25%, d=0.91.
7) Tris buffer; Tris Aristar - 45205, pH 9.6.
8) Borate buffer; Boric acid, Analar, BDH-10058, in DDW and pH adjusted with 5N NaOH.
9) Dopamine; Kochlight 3283-00 in 0.1N HCl.
10) Adrenaline; L-adrenaline hydrogentartrate, Kochlight

977.60 in O.01N HCl.

11) Noradrenaline; L-noradrenaline bitartrate, Kochlight 4351.70 in O.01N HCl.

12) Magnesium Chloride; 500 mmolar, Merck $MgCl_2.6H_2O$.

13) Dithiotreitol; A grade, Calbiochem.

14) Enzyme mix: 1mg DTT (13)
500µl Tris (7)
COMT (prepared)
$MgCl_2$ (12)
SAM (1)

15) Carrier solution:

3-methoxytyramine	30.5mg
D,L-metanephrine	29.6mg
D,L-normetanephrine	30.0mg
HCl, O.01N	up to 25ml.

16) TLC tank solvent, per tank:

Chloroform	80ml
Ethanol p.a.	15ml
Ethylamine	10ml

17) Scintillation solution B:

Butyl-PBD	12.5g
Toluene	2.5l

18) Elution solution for methoxytyramine:

Triton X-100	20g
Acetic acid	100ml
Methanol	up to 1 l

Procedure:

Plasma samples: 1 volume of plasma was deproteinised using 1 volume of 0.6 $HClO_4$, mixed on a vortex mixer and centrifuged for 5 mins. 2mg $NaIO_4$ was added to 1ml of plasma and the catechols oxidised at 35°C for 10 mins. 1ml 0.6N $HClO_4$ was then added and the sample mixed and centrifuged.

Tissue samples: Tissues were excised and frozen immediately in liquid nitrogen. The tissue was then powdered by percussion homogenization and weighed. 10ml per g ice-cold $HClO_4$ was then added and centrifuged in a bench ultracentrifuge for 10 mins at 15000 rpm. The supernatant was decanted and the pellet was re-extracted with another equal volume of $HClO_4$. Extracts were then combined.

Estimations in plasma:

	Blank zero	Oxidation blank	Standard	Internal standard	Sample
$HClO_4$	100	-	100	-	-
Deproteinised plasma	-	-	-	100	100
Oxidised plasma	-	100	-	-	-
Standard	-	-	10-50	50	-
0.01 N HCl	50	50	40-0	-	50
Enzyme mix	100	100	100	100	100

Incubated for 1 hour, continually shaken slowly.

Estimations in tissue:prepared on ice

	Blank zero	Standard	Internal standard	Sample
$HClO_4$	100	100	-	-
Extract	-	-	100	100
Standard	-	50	50	-
0.01N HCl	50	-	-	50
Enzyme mix	100	100	100	100

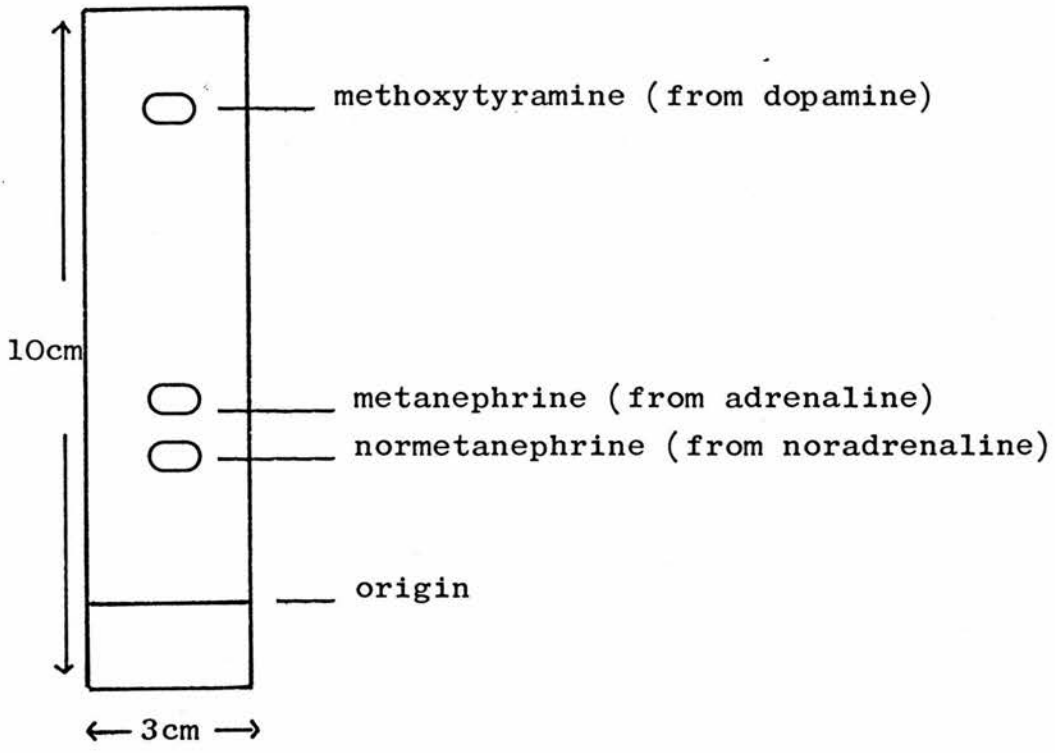
Extraction of 3-O-methylated products from the reaction mixture:

Tubes were cooled on ice.
200µl of 3 parts borate buffer to 1 part carrier solution, freshly-prepared added.
100µl sodium tetraphenylborate added.
Catechols were extracred into 10ml diethylether.
Tubes were shaken for 5 mins and centrifuged at 1500 rpm for 5 mins.
The water phase was frozen on dry ice.
The ether phase was transferred and 0.5ml 0.1N HCl added.
Tubes were shaken for 5 mins and the water phase frozen on dry ice.
The ether phase was discarded.
The acid phase containing the methylated products were washed with 5ml butylacetate and shaken for 5 mins and then recentrifuged at 1500 rpm for 5 mins.
The water phase was frozen on dry ice and the butyl-acetate phase discarded.
The acid phase was taken to dryness under high vacuum.
The residue was dissolved in 0.01N HCl.

Separation of the amines:

50µl of the purified extract was spotted onto 3 x 20 cm pre-cut TLC plates with a preadsorbed origin (LK5F, Whatman).
105ml of elution solution was equilibrated in a TLC tank.
12 plates were eluted simultaneously in one tank for approximately 1 hour. The TLC plates were dried by exposure to a

stream of cold air. Regions of catecholamines were identified under minimum exposure to UV light.



Elution of methoxytyramine:

The region corresponding to the appearance of methoxy-tyramine was scraped carefully with a blade and transferred with the aid of greaseproof paper to a liquid scintillation vial. The methoxytyramine was eluted by the addition of 0.5ml 0.1N HCl and shaken for 20 mins. 10ml of NE260 scintillant was added to each vial, vortex-mixed and counted.

Elution and oxidation of metanephrine and normetanephrine:

The two regions were scraped separately with a blade and transferred to liquid scintllation vials with the aid of greaseproof paper. 1ml 2N ammonia was then added and the vials shaken for 5 mins. 50µl fresh sodium periodate

solution was added and the vials shaken for 5 mins. The oxidation was stopped by the addition of 50µl glycerol 10% v/v solution and shaken for 5 mins. 500µl acetic acid, 1ON, was added to readjust the pH. 10ml scintillation solution B was added. The vials were capped and shaken vigorously for 20 mins to extract the oxidised products into the toluene. All vials were then counted for 20 mins.

5. Preparation of guinea-pig cardiac membranes for binding studies:

Guinea-pig hearts were excised as quickly as possible after death. Fat, pericardium and great vessels were dissected free and the ventricles and atria separated. The tissues were washed in ice-cold sucrose buffer (0.25M sucrose, 5mM $MgCl_2$, pH7.4). The tissues were minced finely, weighed and homogenized in 10 volumes ice-cold sucrose buffer with 10 strokes of a hand-held Potter-Ehlvehjem glass homogenizer. The homogenate was filtered through two layers of nylon gauze then centrifuged at 480g for 10 mins at 4°C. The supernatant was diluted with an equal volume of 1M KCl to break down contractile proteins and left on ice for 10 mins. The supernatant was then re-centrifuged at 48000g for 10 mins at 4°C. The resulting membrane pellet was then carefully resuspended in Tris buffer (50mM Tris, 10mM $MgCl_2$,pH 7.5) and kept at 4°C if to be used at once or frozen in liquid nitrogen if to be stored for later use. Protein concentrations were measured by the method of Lowry et al (1951).

Preparation of ^{125}I-pindolol:

10µl 13.5mM HCl containing 20µg (-)pindolol, 20µl 0.3M potassium phosphate buffer (pH 7.6), 1mCi Na^{125}I (as the carrier-free compound) and 20µl aqueous solution of chloramine T (o.34 mg/ml) were combined in that order in a polypropylene test tube and allowed to stand at room temperature for 5 mins. 300µl aqueous $Na_2S_2O_5$ solution (1mg/ml) was added to stop the reaction. 10µl 1N NaOH was finally added. The iodinated product was then extracted 4 times with 300µl portions of ethylacetate containing 0.01% phenol. Phase separation was carried out in a Pasteur pipette. The 4 washes were combined and spotted onto a 25cm x 40cm strip of Whatman 3MM paper and were chromatographed in a descending manner at 4°C with 0.1M ammonium formiate (pH 8.5) containing 0.01% phenol for 4 hours. The chromatograph was removed from the tank and cut into 1cm strips while still wet. Each strip was washed in 5ml methanol. The ^{125}I-pindolol forms in a single narrow peak approximately 7cm from the origin and is separated well from the remaining unconverted pindolol. The separate fractions were counted to detect the purest peaks of radioactivity and these fractions were combined. The solution was dried down under nitrogen flow to give a volume of 1ml and stored at -20°C until required.

Binding studies:

Serial dilutions of ^{125}I-iodopindolol (200µl) were incubated with 300µl aliquots of guinea-pig atrial or

ventricular membranes to construct a binding isotherm. The non-specific binding was determined on all occasions by the use of a saturating concentration (200μM) of isoprenaline. NSB was plotted against concentration of radioligand to ensure that it was constant. Scatchard analysis of the radioligand binding (specific bound/free versus specific bound) gave an estimate of the receptor numbers - B_{max}. Displacement of specific ^{125}I-iodopindolol binding was determined by incubation of a fixed amount of radioligand with serial dilutions of the competing cold ligand. The resultant displacement achieved gave an estimate of the cold ligand's potency. GTP nucleotides are known to produce an alteration of high affinity binding sites to low affinity binding sites for β-adrenoceptors (Kent et al, 1979) and 10^{-4}M Gp(NH)pp was added to a series of displacement binding studies to determine the proportion of the two contributing affinity binding states of the β-adrenoceptor. Kinetic binding experiments were carried out as above but at varying incubation times to determine the rate constants for the ligand-receptor 'on' and 'off' reactions.
All assays contained $5x10^{-4}$M ascorbic acid. Incubations were carried out at 37°C and were terminated by rapid vacuum filtration over Whatman GF/C filters and washed with 3 x 4ml Tris buffer. Radioactivity bound to the filters was measured at 80% efficiency.

Data analysis:

B_{max} values were determined by weighted least squares fit regression analysis. Binding data was fitted by a computer

modelling programme based upon the law of mass action, and allowing for multi-compartmental systems of β-adrenoceptors (Minneman et al, 1979; De Lean et al, 1981). Initial analysis was carried out by hand to check that there was agreement.

6. Conscious Dog Experiments:

Male foxhounds (22-23 kg) were trained to lie on their side and remain still throughout a 45 minute test period. ECG recordings were made with metal plate electrodes lubricated with saline jelly as a conductant and strapped to the underside of each limb on a shaved area. Recordings were made on an Elctromed devices heat pen recorder. Heart rate, PR and QT intervals were made at the isoelectric point. Drug infusions and injections were made via an intravenous 'butterfly' cannula inserted into the right radial vein. The β-blocking potency of each antagonist employed was assessed by its ability to shift the log dose - response curve of heart rate increases to injected isoprenaline to the right. Bolus injections of isoprenaline were made into a giving set attached to a sterile saline drip and the heart rate monitored until the peak response had occurred. Several doses of the agonist were given at 5 minute intervals. The antagonist was then injected and after 10 minutes the curve was repeated. The parallel shift in the log dose - response curve was taken as an estimate of tne β-blocking potency of the antagonist. Since all of the antagonists employed in the study were known to be non-

selective β-blockers, it was estimated to be a sufficient index without a block of vagal tone eg. by atropine. Antagonist infusions were given over a 30 minute test period at an infusion rate of 1ml/min on a Watson - Marlow infusion pump. ECG recordings were taken at regular intervals throughout the study period, from Lead II.

Alteration of thyroid state:

Dogs were rendered hyperthyroid by oral administration of 3.3 mg/kg triiodothyronine (T_3) in gelatine capsule form twice daily. Hypothyroid state was produced by oral administration of 1mg/kg carbimazole in gelatine capsules twice daily. The desired state was considered to have been achieved when the gross electrophysiological changes associated with that state were observed in the ECG traces that were monitored daily. Alterations in behaviour and physical appearance gave support to the ECG findings. The agonist and antagonist dose response curves and infusions were all repeated in each state and total recovery was permitted between the two treatments. Blood samples were taken at regular intervals throughout the treatment cycles in order to monitor plasma T_3 and T_4 levels which were measured by radioimmunoassay.

Expression of results:

For heart rate responses, increments are expressed as % increase above resting levels.

Log dose - response curves of the rate responses to isoprenaline before and after antagonists were used as an index of their β-blocking potency. The resultant

parallel shifts of the log dose - response curves were used to assess the relative blocking potencies of the different antagonists. These results were then used to correct the infused concentrations so as to express all antagonists in the form of their Dose Ratio (i.e. equating doses of different antagonists which would achieve an equal block of response to an agonist). This was in order that results could be expressed in such a way as to separate β-blocking effects from any other properties of the drugs so that any differences between the individual β-blockers would be more easily apparent.

7. Anaesthetised rats:

Wistar rats of either sex with an average weight of 300g were anaesthetised with an intraperitoneal injection of 25% urethane (0.7mg/100g). Incisions were made and any bleeding points coagulated using an Endofrex electro-surgical unit. The trachea was cannulated and the rat ventilated with air using a variable stroke Starling 'ideal' pump running at 70 strokes per minute. A jugular vein was cannulated for the administration of solutions via a small volume Palmer injection apparatus. Temperature was held at 37°C by means of a heating pad and infra red lamps as required. A common carotid artery was cannulated in order to take 100µl samples of blood at 5-minute intervals throughout the experiment. Blood gas tensions were measured on a Blood Micro System connected to an acid-base analyser (Radiometer, Copenhagen). The system was calibrated with a high and low concentration

of gases passed from a calibration cylinder and bubbled through a humidifier and past the detector electrode membranes at 37°C. The low concentration gas mixture was 3% CO_2 in 97% N_2 and the high concentration was 10% CO_2 in 40% N_2 and 50% O_2. The relative partial pressures of the gas mixture were corrected for water vapour content and atmospheric pressure (AP) by the formula

$$\text{partial pressure gas X} = \frac{\text{AP} - 49}{100} \times \%\ \text{gas X}$$

ECG records from lead II were recorded with a capacitatively coupled amplifier passing a band between 50 and 70 Hz. Results of the β-blocker infusions in the ventilated and and unventilated rat were expressed in terms of dose ratios extrapolated from the previous dog data since it was found to be impossible to evoke a heart rate response to isoprenaline in the anaesthetised rat.

8. Expression of results:

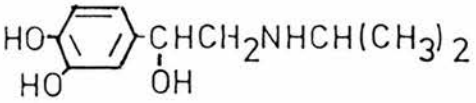
Unless otherwise stated, results are expressed either as absolute changes or as percentage changes from control values. Where results have been pooled, each value is an expression of the mean of the pool and standard deviations are given where appropriate, and number of observations given. Regression analyses were carried out by the least squares fit method and 95% confidence limits for the regression lines are shown if two regression lines are significantly different from each other. Student's 't'-test, Wilcoxon rank tests or an analysis of variance were used to calculate the significance of results, changes being considered significant at the 1% or 5% level.

9. Drugs used:

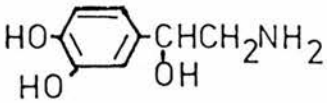
The chemical structures and forms of the drugs used in the present study are given with their suppliers in Fig,1.

FIGURE 1

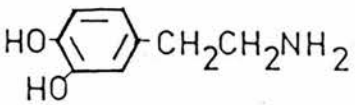
DRUGS USED IN THE STUDIES.



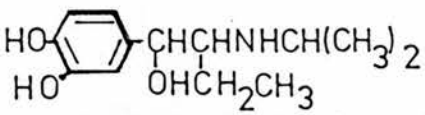
ISOPRENALINE (Sigma)



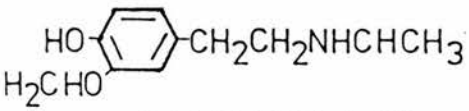
NORADRENALINE (Sigma)



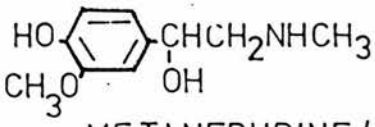
DOPAMINE (Kochlight)



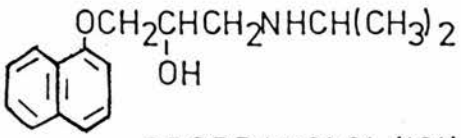
ISOETHARINE (Riker)



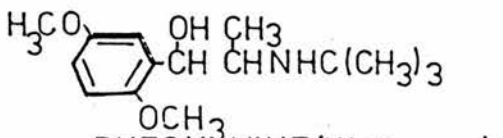
SALBUTAMOL (Sigma)



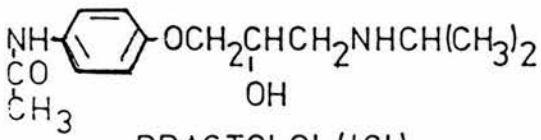
METANEPHRINE (Sigma)



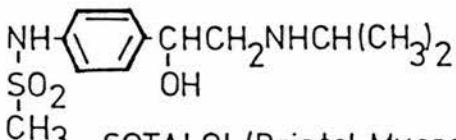
PROPRANOLOL (ICI)



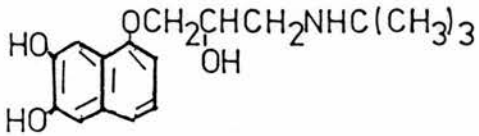
BUTOXAMINE (Wellcome)



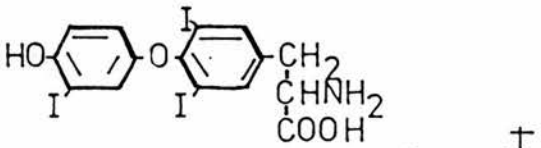
PRACTOLOL (ICI)



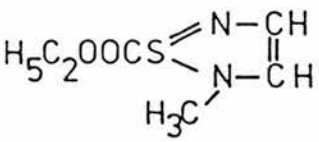
SOTALOL (Bristol-Myers)



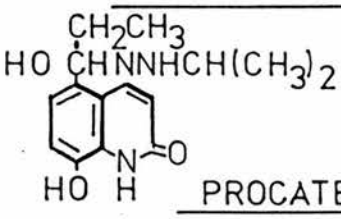
NADOLOL (E.R.Squibb)



TRIIODOTHYRONINE (Sigma) †



CARBIMAZOLE (Nicholas)



PROCATEROL (Otsuka)

Erythro-DL-1-(7-methylindan-4-yloxyl)-3-isopropylamino-butan-2-al ICI 118 551 (ICI)

1-(5-chloracetylaminobenzfuran-2-yl)-2-isopropylamino-ethanol Ro-03 7894 (Roche)

All drugs used in HCl form except † as Na salt.

SECTION III : RESULTS

The effects of 6-hydroxydopamine pretreatment on the catecholamine stores of the guinea-pig heart.

The effects of 6-OHDA pretreatment on the stores of catecholamines of the guinea-pig heart were examined by two different experimental means: a) autofluorescence microscopy and b) radioenzymatic determination.

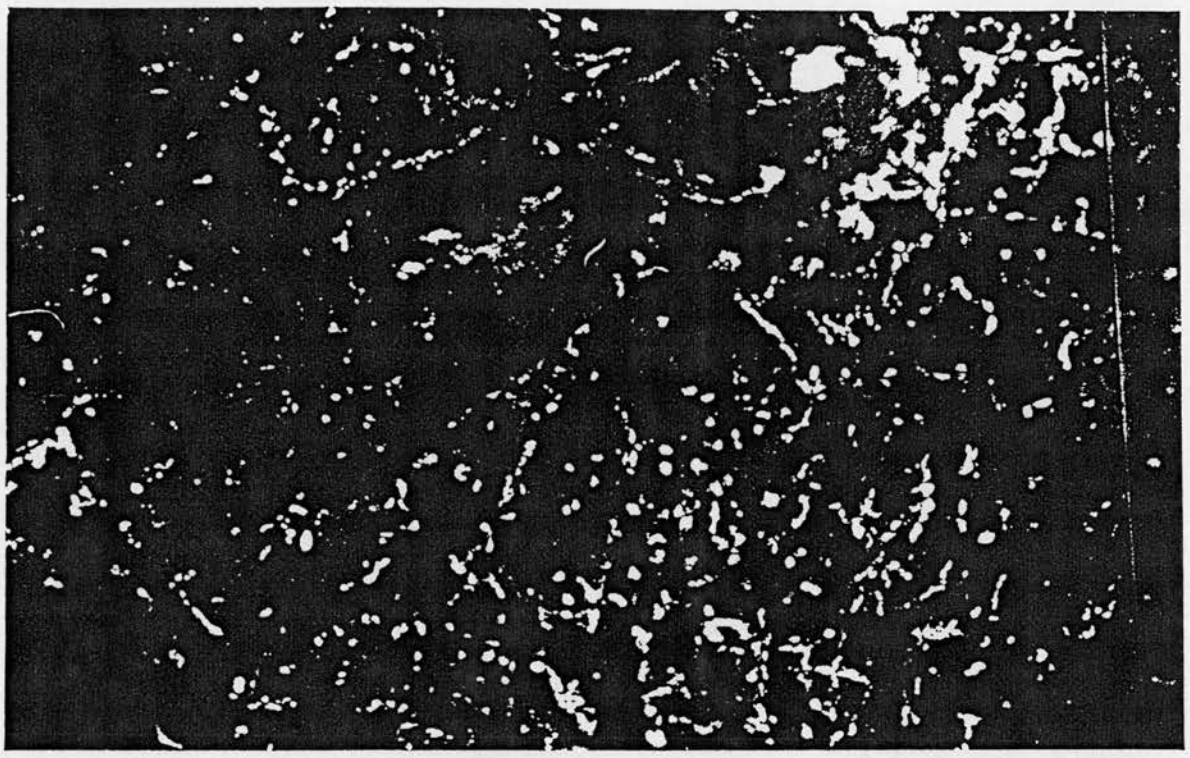
Autofluorescence microscopy:

This investigation was carried out according to the sucrose-phosphate-glyoxylic acid (SPG) method of de la Torre and Surgeon (1976) because of its simplicity, sensitivity and stability. The resulting photographs are shown in Figs. 2 and 3. Figs. 2a) and b) show the alteration in catecholamine fluorescence brought about by 6-OHDA pretreatment of guinea-pig atria while Figs 3a) and b) show the corresponding alteration in guinea-pig ventricles. It is obvious that there is a significant gross reduction in fluorescence in the denervated organs. The strings of fluorescent vesicles seen in the control photographs were consistently absent from the chemically-denervated tissues. It is possible that a small component of the remaining fluorescence is due to a cross-reactivity with serotonin-containing cells or be due to residual dopamine-containing stores which may not be depleted to the same extent as the other catecholamines.

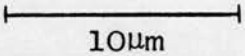
Radioenzymatic determination:

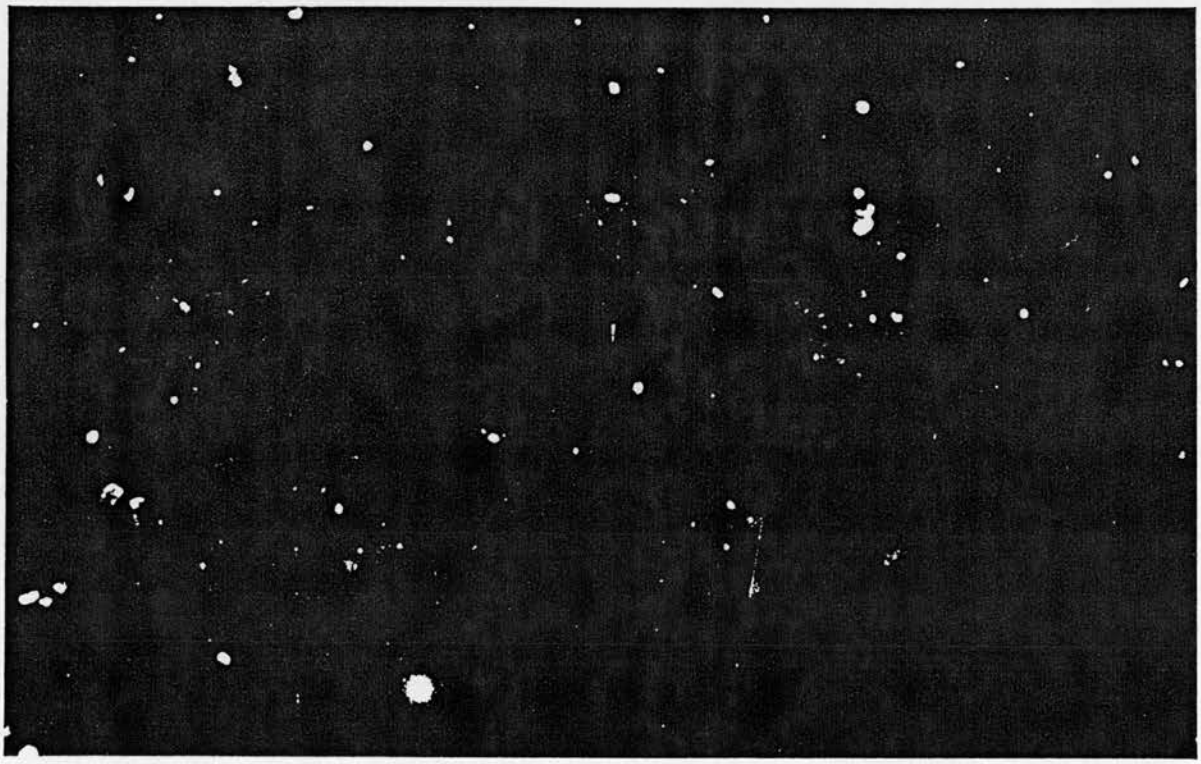
The method used to quantify the catecholamine content

FIGURE 2



(a)

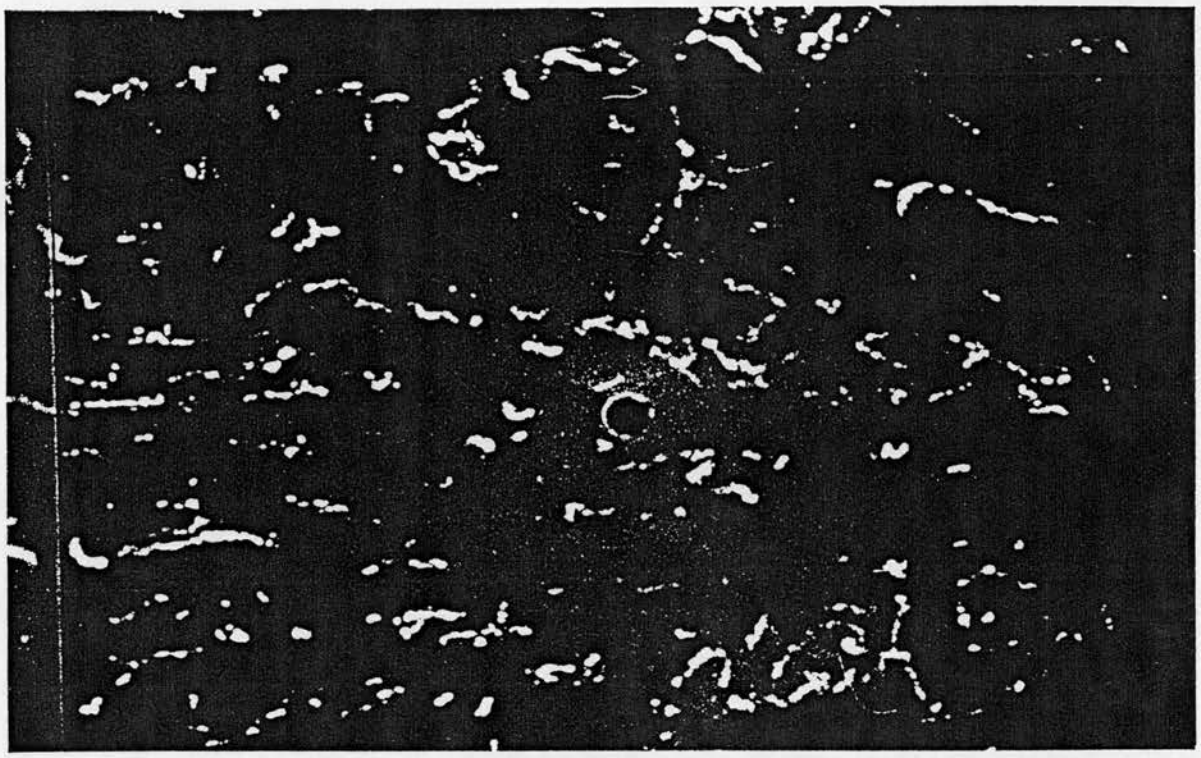




(b)

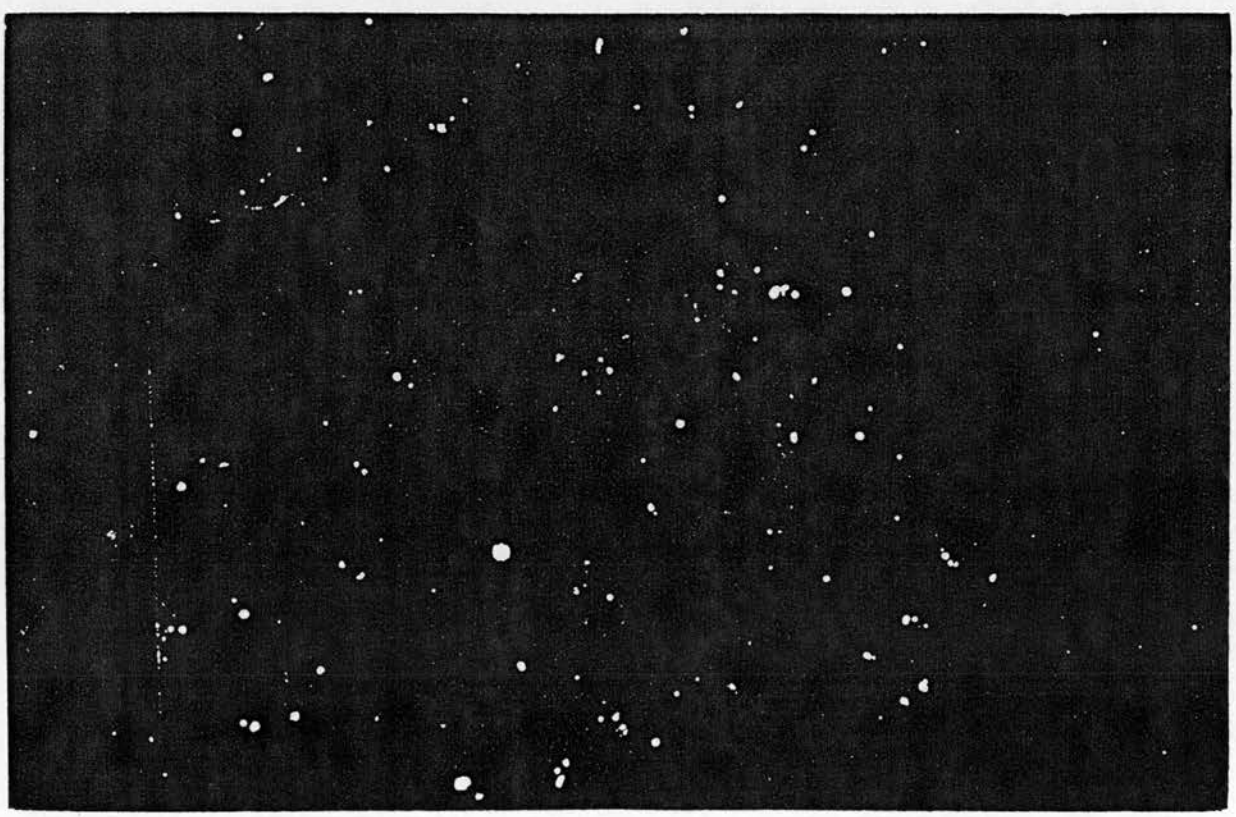
Autofluorescence micrographs of atrial catecholamines observed by the SPG method in the control (a) and the 6-hydroxydopamine - sympathectomized (b) guinea-pig.

FIGURE 3



(a)

10µm



(b)

Autofluorescence micrographs of ventricular catecholamines observed by the SPG method in the control (a) and the 6-hydroxydopamine - sympathectomized (b) guinea-pig.

of the control and denervated hearts was a radio-enzymatic method modified from that of Da Prada and Zurcher (1976). This method was used to quantify both cardiac and circulating plasma levels of adrenaline, noradrenaline and dopamine. The results may be seen in Table 1. As was expected, a control cardiac NA:Ad ratio of approximately 10:1 was observed and this was not significantly altered in the denervated organ. In the heart, both Ad and NA were reduced by approximately 97% while the reduction in DA content was not large but was still significant at approximately 30% reduction. The plasma levels of catecholamines indicated a higher proportion of Ad as expected from a basal adrenal release. Plasma catecholamines proved to be more resistant than cardiac catecholamines to depletion, NA being reduced by 87% and Ad by 53%, no values being available for DA. This result may be due to a 'buffering' effect of catecholamines released from regions more resistant to depletion by 6-OHDA, possibly related to the length of the sympathetic postganglionic axon, blood supply etc such as in the genito-urinary tract or the sex organs, though, in fact, may be accounted for solely by elevated release from the adrenals if there was no significant alteration of release from other 'resistant' organs (de Champlain and van Ameringen, 1973). This is indeed indicated by the decreased NA:Ad ratio .

TABLE 1

THE EFFECTS OF 6-HYDROXYDOPAMINE PRETREATMENT ON CARDIAC AND CIRCULATING CATECHOLAMINE LEVELS.

AMINE	CONTROL CARDIAC µg/mg wet weight-tissue	6-OHDA CARDIAC µg/mg wet weight-tissue	%reduction	CONTROL CIRCULATING ng/ml plasma	6-OHDA CIRCULATING ng/ml plasma	%reduction	RATIO NA:A CARDIAC	RATIO NA:A PLASMA
NA	1·55	0·041	97·4	16·76	2·17	87	CONTROL 90.2:9.8	CONTROL 60·8:39·2
A	0·15	0·004	97·5	10·79	5·07	53	6 OHDA 91·1	6 OHDA 30·1:69·9
DA	0,045	0.032	29.8	--	--	--	--	--

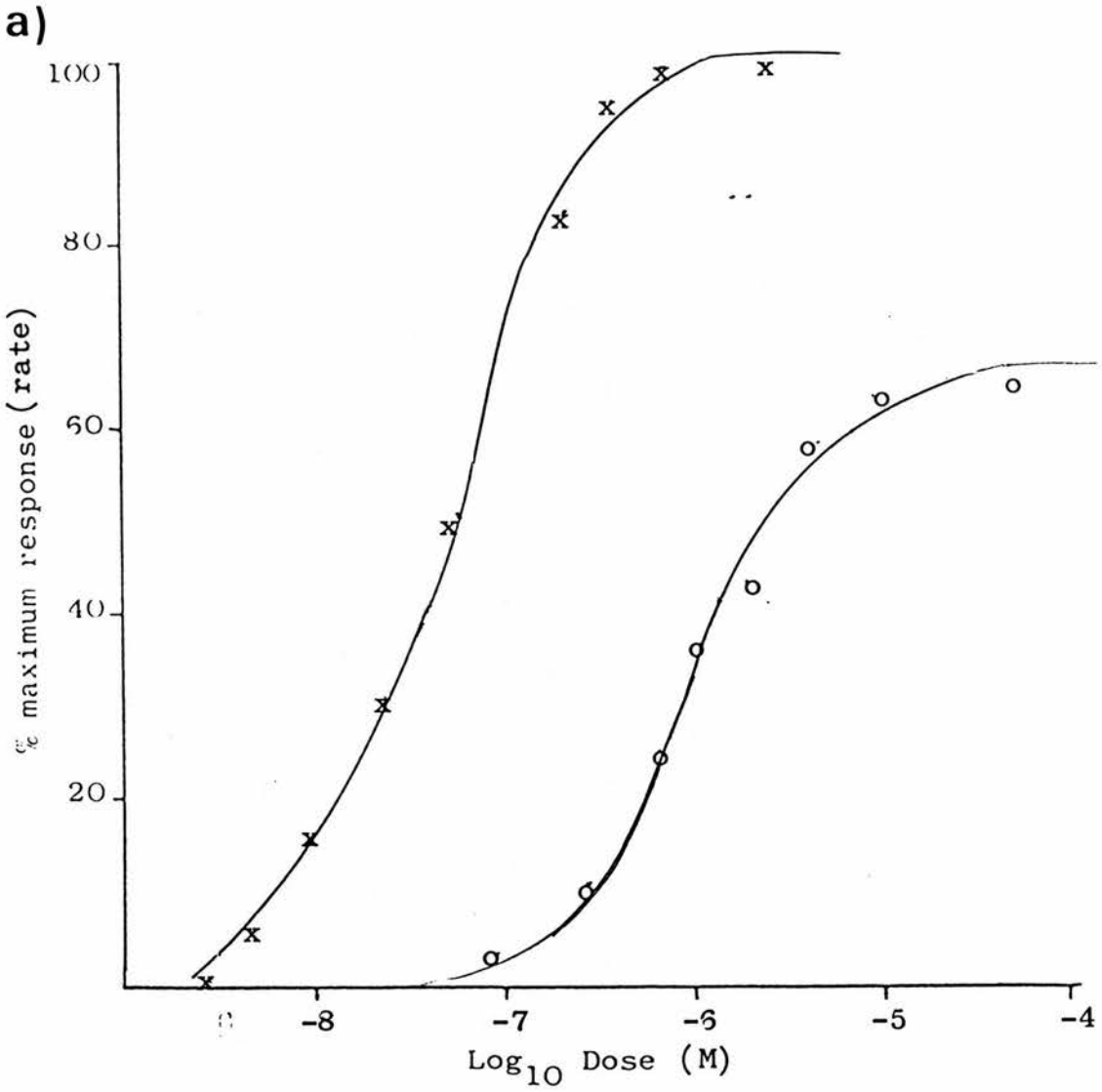
The effects of chemical denervation on responses of isolated guinea-pig atria to adrenergic stimuli.

6-OHDA pretreated guinea-pigs appeared outwardly normal and exhibited no overt signs of distress. Guinea-pigs pretreated with reserpine, however, appeared listless and lethargic within 24 hours of the first intra-peritoneal injection and this was often accompanied by impairment of muscular coordination.

Control atria.

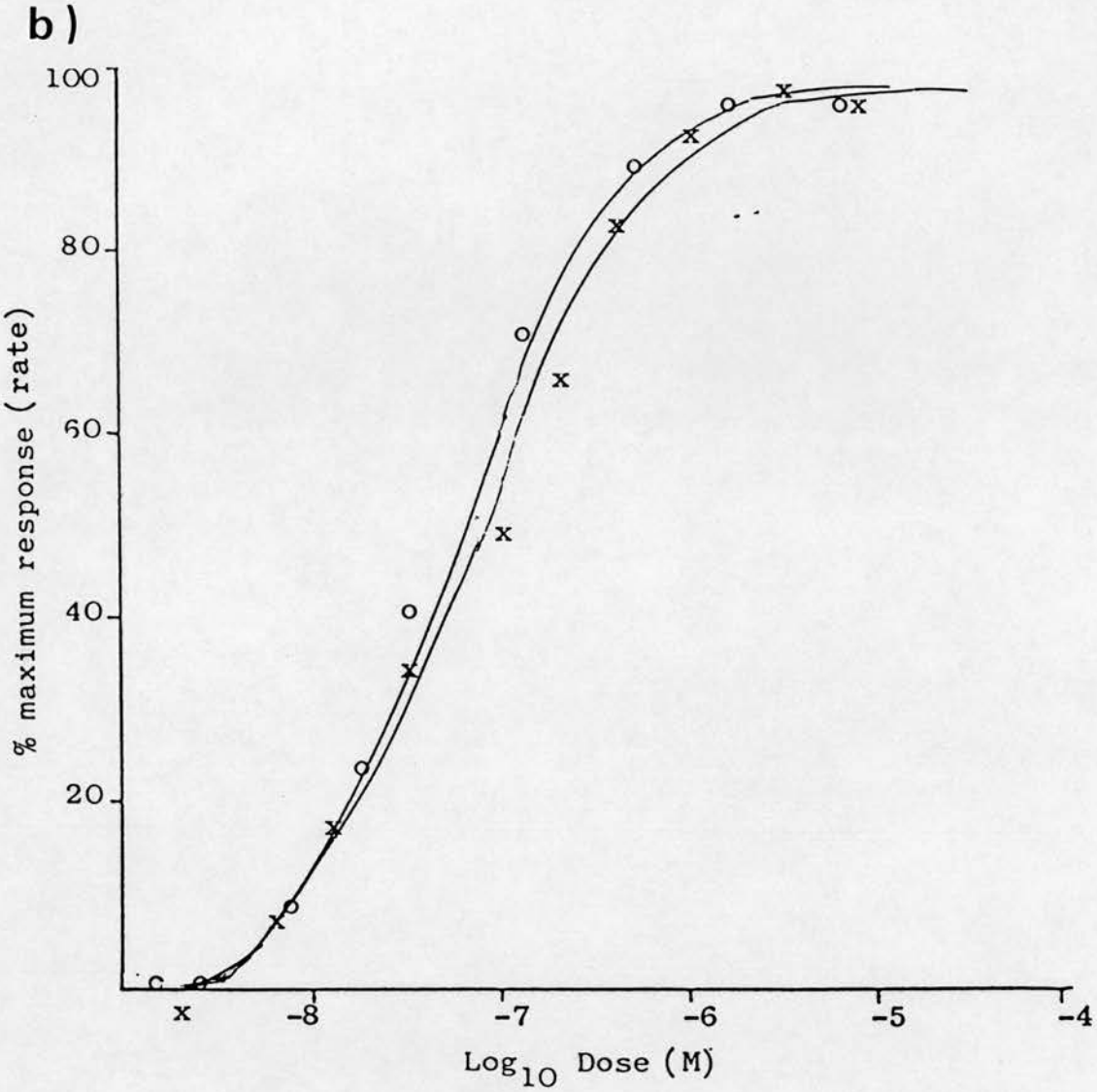
The effects of a series of agonists and antagonists were examined on both spontaneously-beating and electrically-driven atria in order to separate the inotropic and chronotropic effects of the drugs as far as possible. All drug additions were carried out in duplicate. Fig 4a shows a typical comparison of the effects of isoprenaline and salbutamol on the chrono-tropic response of right atria. Salbutamol acted to produce a sub-maximal response when measured against isoprenaline, evoking a 65% maximum chronotropic and a 55% maximum inotropic response, previously described as partial agonism (Broadley and Lumley. 1977). Fig 4b demonstrates a similar comparison of the effects of NA and isoprenaline (ISO). NA evoked a maximal chrono-tropic and inotropic response when compared to ISO and was of the same order of potency. Fig 5 shows the very weak positive chronotropic effect of 6-OHDA itself. The effect was also observed as an inotropic response of the driven atria but was also extremely

FIGURE 4



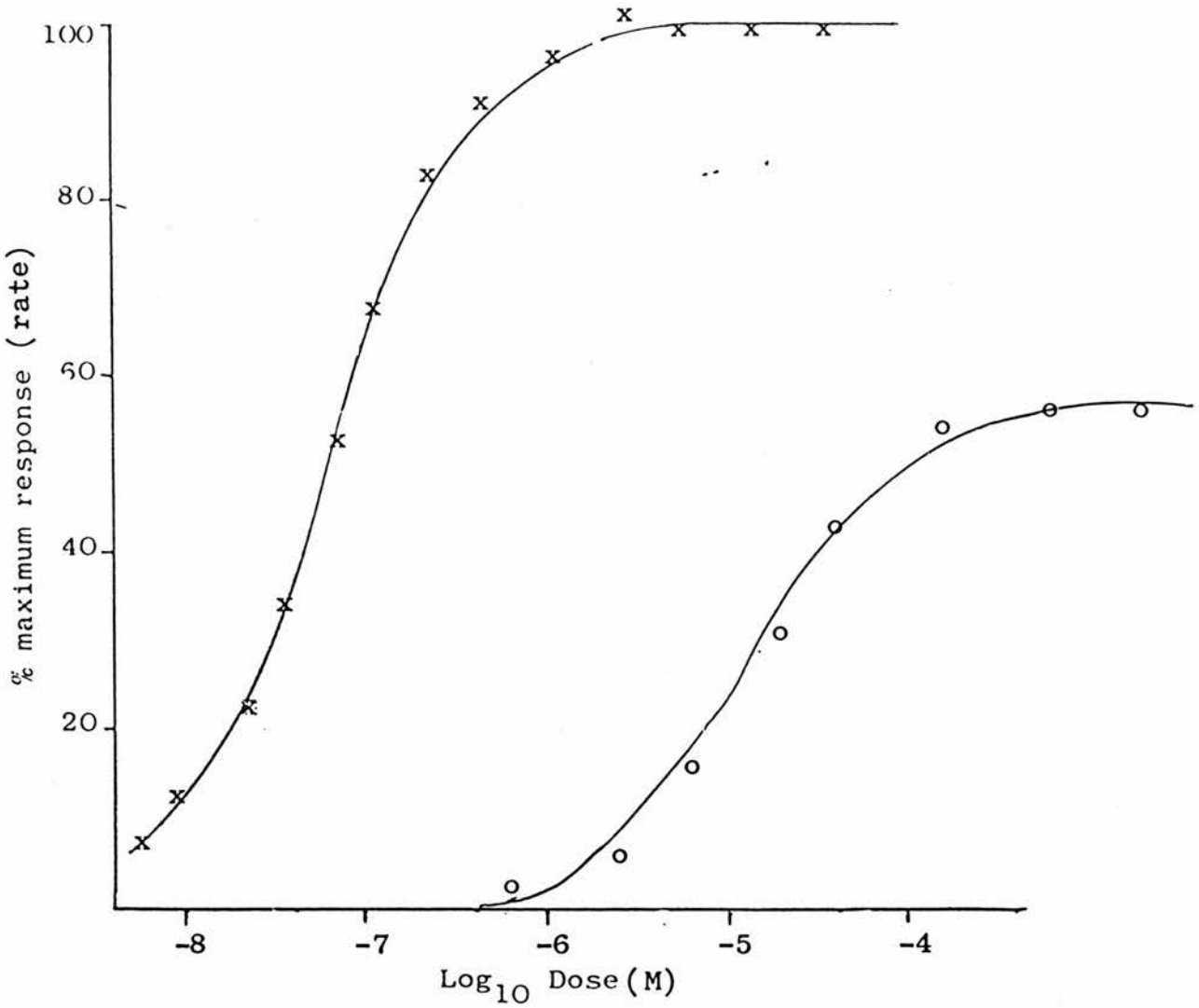
Log dose - response curves showing the relative effects of cumulative doses of isoprenaline (x) and salbutamol (o) on the rate responseof control spontaneously-beating guinea-pig atrium.

FIGURE 4



Log dose - response curves showing the relation between cumulative doses of isoprenaline (o) and noradrenaline (x) on the control spontaneously-beating guinea-pig atrium.

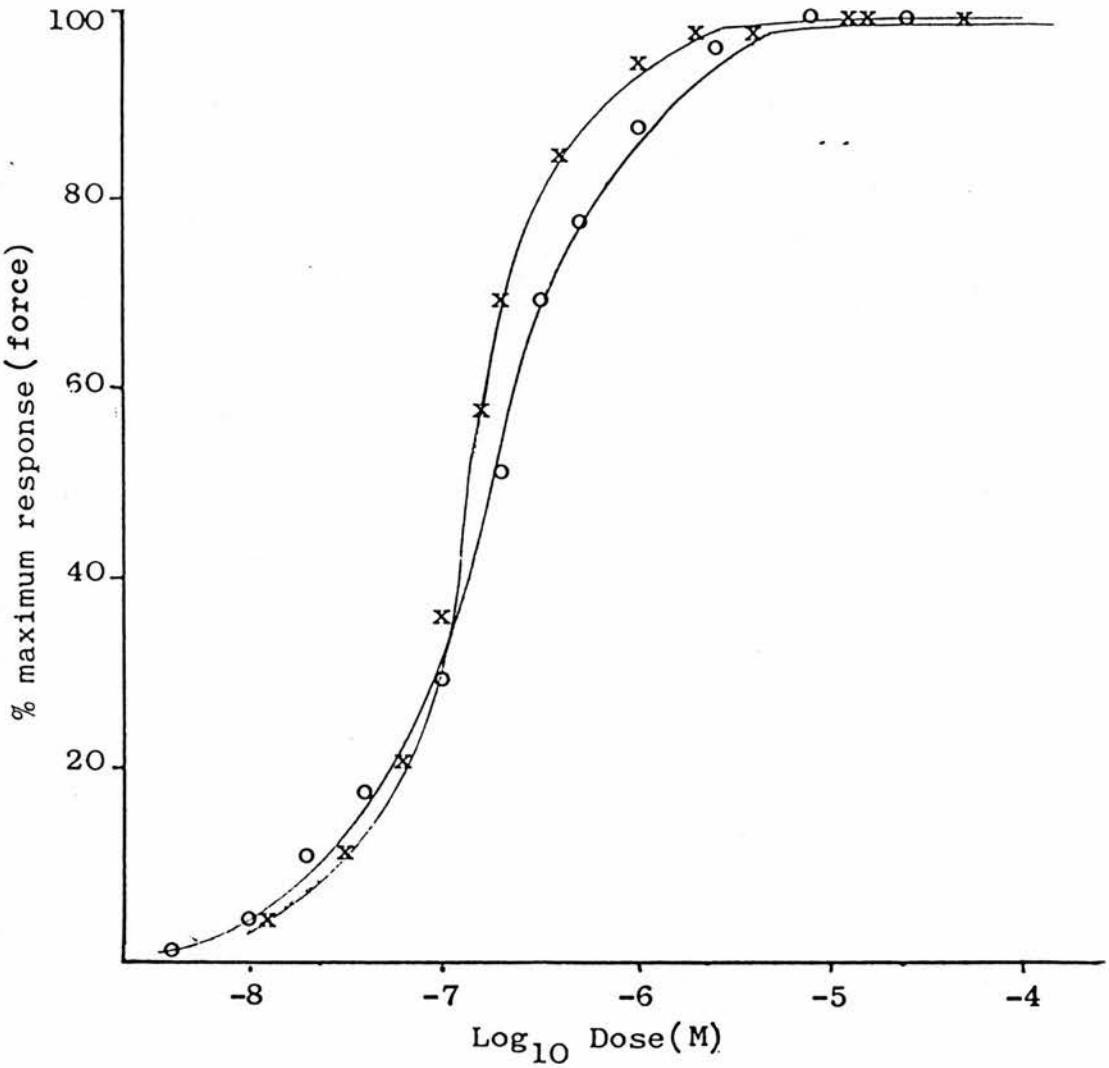
FIGURE 5



Log dose - response curves showing the relationship between the rate responses of the control free-running guinea-pig atrium to isoprenaline (x) and to 6-hydroxydopamine (o).

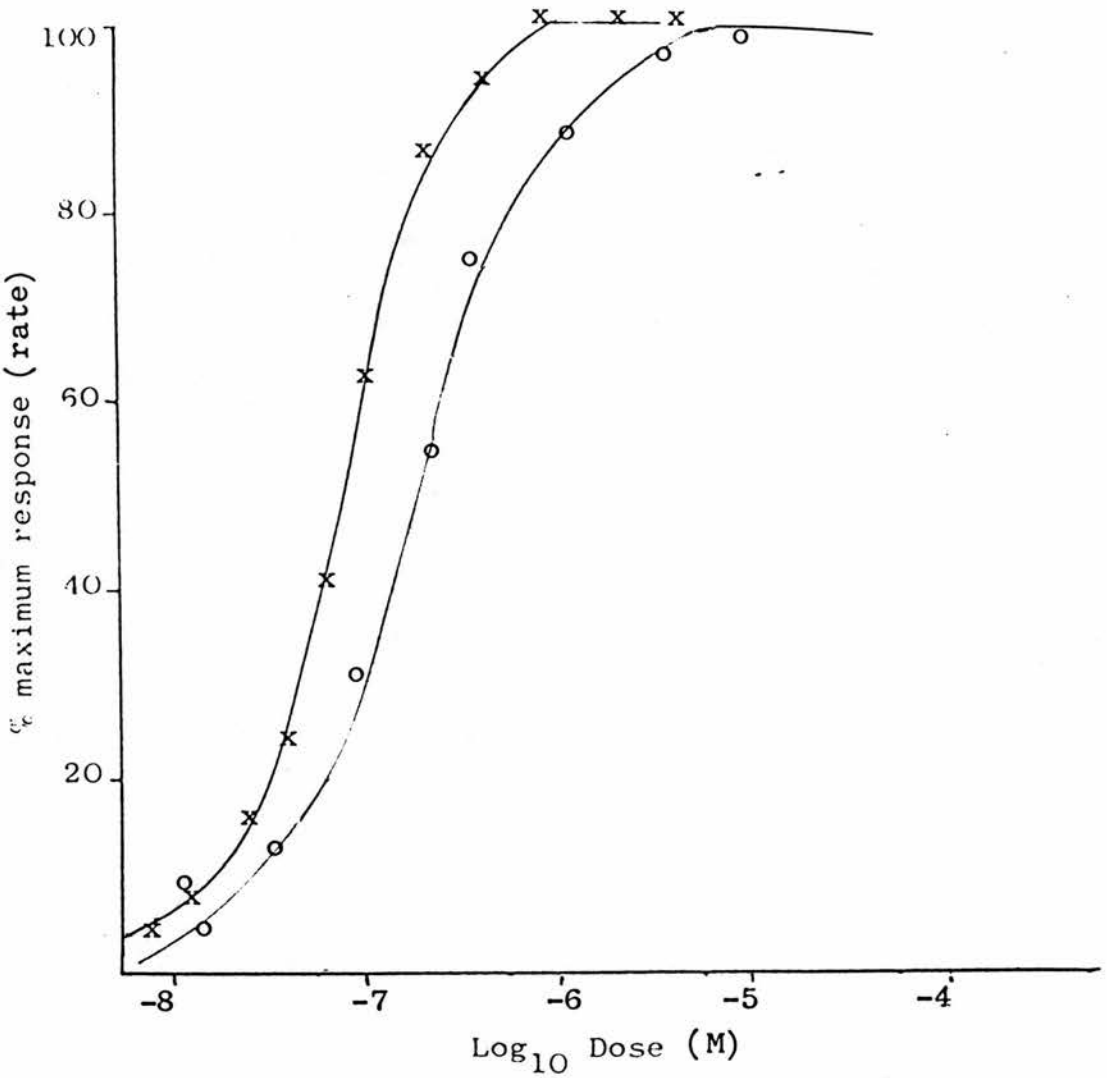
weak, though dose-dependency was evident, the equipotent molar ratio (EMR) for both was approximately 600. In 6-OHDA - pretreated atria, however, 6-OHDA addition to the organ bath evoked no response at all. When 6-OHDA was present in the bathing medium during construction of a dose - response curve to ISO, it was seen that it produced no antagonistic effect of the evoked response at receptor level, see Fig 6. The addition of antagonists produced shifts in the log dose - response curves of right and left atria to the agonists employed. Fig 7 shows an example of the parallel shift produced in the log dose - response curve to ISO by the 'cardioselective' antagonist practolol. This β_1-selective antagonist behaved in a similar manner when used against NA and salbutamol (Salb) indicating a first - order competetive antagonism of the positive inotropic and chronotropic response to an adrenergic stimulus. Although Salb was shown to consistently produce a submaximal response in control atria, this response was still susceptible to shifting by antagonists, eg. by the non-selective irreversible antagonist Ro-03 7894, but its 'maximum' effect was depressed, see Fig 8. All values measured are shown in Table J. The order of potency for practolol (β_1-selective) was NA > ISO > Salb; for Ro-03 7894 (non-selective) the order was ISO > NA > Salb; for ICI 118 551 (β_2-selective) the order was Salb > ISO > NA, all measurements in the rank differing significantly from each other at the 5% significance level.

FIGURE 6



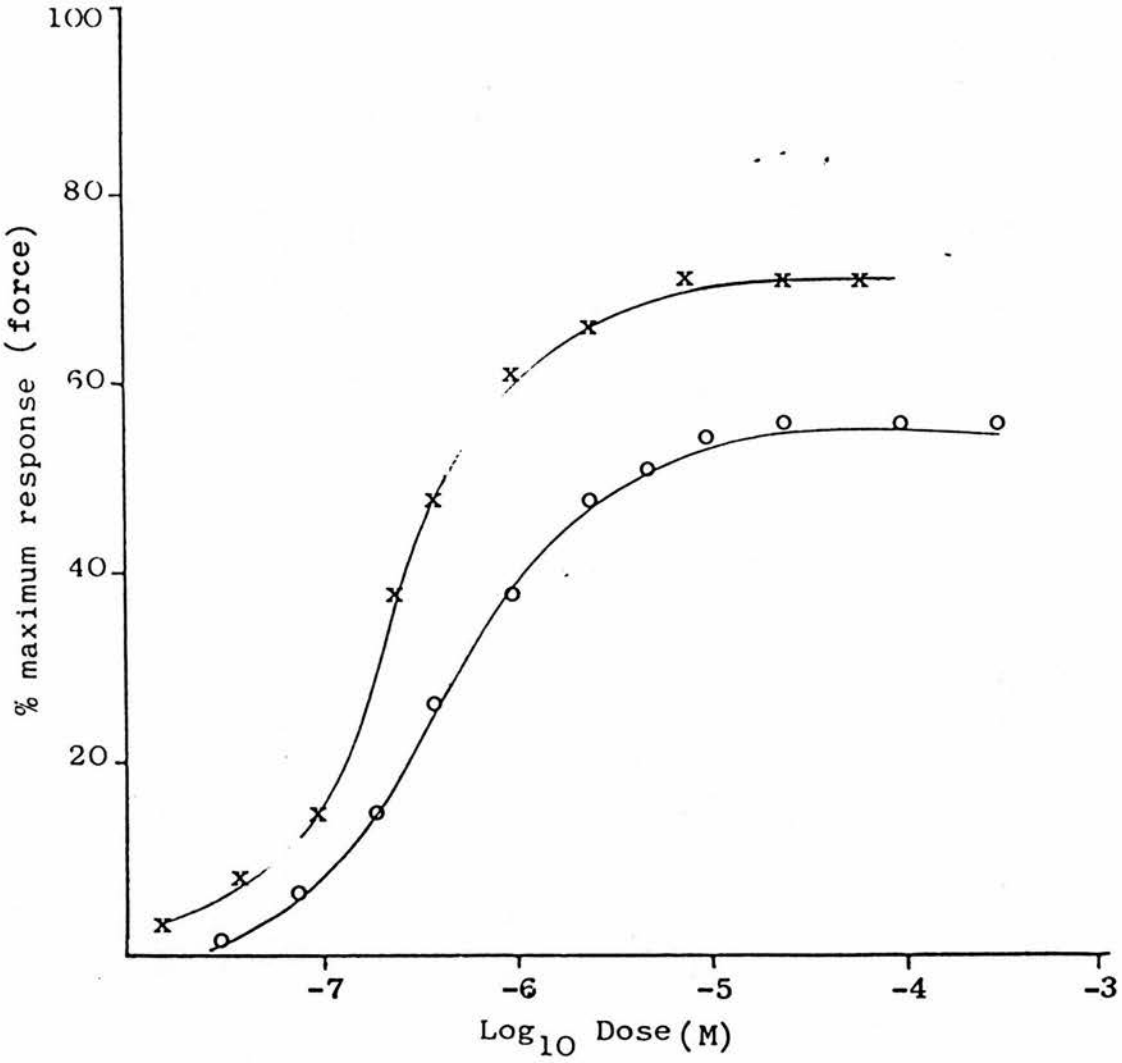
Log dose - response curves showing the effect of cumulative doses of isoprenaline (x) on the tension response of the electrically-driven control guinea-pig atrium in the absence and presence of 10^{-5}M 6-hydroxydopamine (o). The curves are not significantly different.

FIGURE 7



Log dose - response curves showing the effect of cumulative doses of isoprenaline (x) on the rate response of the spontaneously-beating control guinea-pig atrium and the parallel shift produced in the presence of 10^{-6}M practolol (o).

FIGURE 8



Log dose - response curves showing the sub-maximal effect of salbutamol when compared to isoprenaline (100%) on tension responses of the electrically-driven control left guinea-pig atrium (x) and the shift produced by the addition of 6.4×10^{-4}M Ro-05 7894 (o).

/

Comparison of control and denervated atria.

Combinations of experiments coordinating selective and non-selective agonists and antagonists were carried out in both control and denervated atria. It is well-documented that a selective supersensitivity of both chronotropic and inotropic responses occurs following reserpine - pretreatment of atria (Broadley and Lumley, 1977). This observation was borne out in the present study. However, no such supersensitivity was observed in the case of chemical denervation by 6-OHDA. The EC_{50} values are given in Table 2. Responses to Salb were also enhanced after reserpine - pretreatment but a supersensitivity to NA was not demonstrated, indeed a decreased sensitivity was noted. On the other hand, responses to ISO and NA were unchanged following 6-OHDA pretreatment. However, responses to Salb could not be evoked after 6-OHDA. Even at doses 1000 times those producing its maximal response in control atria, no response at all was observed. In all cases, adult and newborn - treated atria gave identical results. In all cases, agonists displayed a rate selectivity and this may also be seen in Table 2 from their EC_{50} values. This rate selectivity and order of potency are maintained in both control and denervated atria, ISO > NA > Salb.

While no response was obtained to Salb after 6-OHDA, it in fact proved to be an antagonist of responses to ISO and NA. It produced a parallel shift in the log dose - response curves to the right, being less potent

TABLE 2

AGONIST	CONTROL		RESERPINIZED		6-OHDA - PRETREATED	
	CHRONOTROPIC	INOTROPIC	CHRONOTROPIC	INOTROPIC	CHRONOTROPIC	INOTROPIC
ISOPRENALINE	7.21×10^{-8}	1.35×10^{-7}	7.86×10^{-9} *	4.46×10^{-8} *	7.14×10^{-8}	1.41×10^{-7}
NORADRENALINE	7.69×10^{-8}	4.40×10^{-7}	8.62×10^{-8} *	8.48×10^{-7} *	7.60×10^{-8}	4.48×10^{-7}
SALBUTAMOL	2.04×10^{-7}	4.38×10^{-6}	1.13×10^{-7} *	2.04×10^{-6} *	$>3.4 \times 10^{-4}$ *	$>3.4 \times 10^{-4}$ *

* Significantly different from control, $P > 0.05$ (Mann-Whitney rank test)

EC_{50} values for the inotropic and chronotropic responses of control, reserpinized and 6-OHDA - pretreated guinea-pig atria to a non-selective, a β_1-selective and a β_2-selective agonist.

against the action of ISO, the K_a values being listed along with those of the true antagonists in Table J.

The effects of tyramine were then tested in control and denervated atria to examine the releasable pool of catecholamines in response to an indirectly - acting sympathomimetic amine. Tyramine produced a weak, dose-related chronotropic and inotropic effect in control atria but this action was not seen in reserpinised or 6-OHDA pretreated atria, indicating an absence of the releasable pool. The effects of catecholamine depletion by reserpine on the K_a values of a β_1-selective (practolol) and a non-selective (Ro-03 7894) adrenoceptor antagonist are depicted in Table 3. The affinity constants of the two types of blocker were unchanged when measured against Salb. The K_a values for the non-selective antagonist were decreased for both chronotropic and inotropic responses when measured against the non-selective and the β_1-selective agonist. The K_a values for the 'cardioselective' β_1-blocker practolol were affected in the opposite direction and were increased in both cases.

Table 4 shows the effects of 6-OHDA - induced denervation on the K_a values of a non-selective (Ro-03 7894), a β_1-selective (practolol) and a β_2-selective antagonist (ICI 118 551) against a selective and a non-selective agonist. The values for the β_2-selective and the non-selectiveantagonists remained unchanged but the K_a

TABLE 3

The effects of reserpine - pretreatment on the Ka values of a β_1-selective and a non-selective adrenoceptor antagonist.

ANTAGONIST	AGONIST	RESERPINIZED	
		CHRONOTROPIC	INOTROPIC
Ro 03 7894 (non-selective)	ISOPRENALINE	↓	↓
	NORADRENALINE	↓	↓
	SALBUTAMOL	—	—
PRACTOLOL (β_1- selective)	ISOPRENALINE	↑	↑
	NORADRENALINE	↑	↑
	SALBUTAMOL	—	—

TABLE 4

THE EFFECTS OF 6-HYDROXYDOPAMINE PRETREATMENT ON K_a OF VARIOUS β-ANTAGONISTS VERSUS A NON-SELECTIVE AND A β_1 SELECTIVE AGONIST

ANTAGONIST	AGONIST	6-OHDA NEWBORN		6-OHDA ADULT	
		CHRONOTROPIC	INOTROPIC	CHRONOTROPIC	INOTROPIC
RO 037894 (non-selective)	ISOPRENALINE	——	——	——	——
	NORADRENALINE	——	——	——	——
PRACTOLOL (β_1-selective)	ISOPRENALINE	↑	↑	↑	↑
	NORADRENALINE	↑	↑	↑	↑
ICI 118 551 (β_2-selective)	ISOPRENALINE	——	——	——	——
	NORADRENALINE	——	——	——	——

value for practolol was increased for both chronotropic and inotropic responses, both adult and neonatal tissues maintaining their identical responses.

The effects of 6-hydroxydopamine pretreatment on the responses of guinea-pig trachea to adrenergic stimuli.

The effect of a series of agonists and antagonists on the relaxation responses of guinea-pig tracheal strips that had developed spontaneous tone were examined. All drug additions were carried out in duplicate.

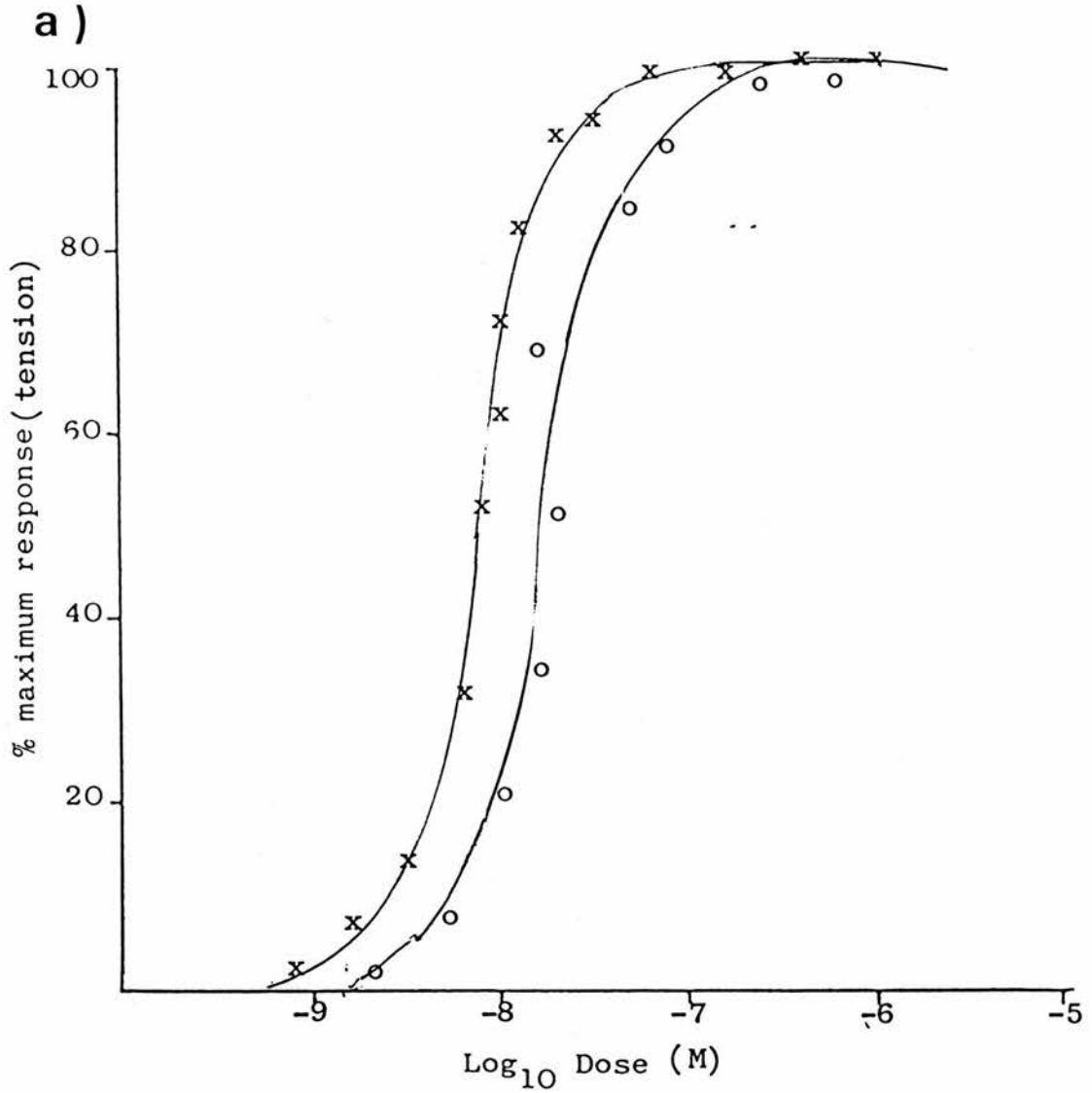
Both non-selective ISO and β_2-selective Salb proved to be full agonists in the trachea. Fig 9a shows an example of the parallel shift to the right of the log dose - response curve obtained to ISO produced by the addition of the β_2-selective antagonist butoxamine. Fig 9b shows a similar case for the shift of the full agonistic effect of Salb by β_2-selective ICI 118 551, also being of an exactly parallel nature. A summary of the EMR and K_a values for all of the drugs employed are shown in Table K.

In control tracheae, the β_2-selective agonist procaterol was more potent than Salb, the EMR of procaterol (PROC) measured against a value of 1 for ISO being 1.93, that of Salb being 3.01.

6-OHDA pretreatment had no effect on the ability of tracheal spiral strips to develop spontaneous tone.

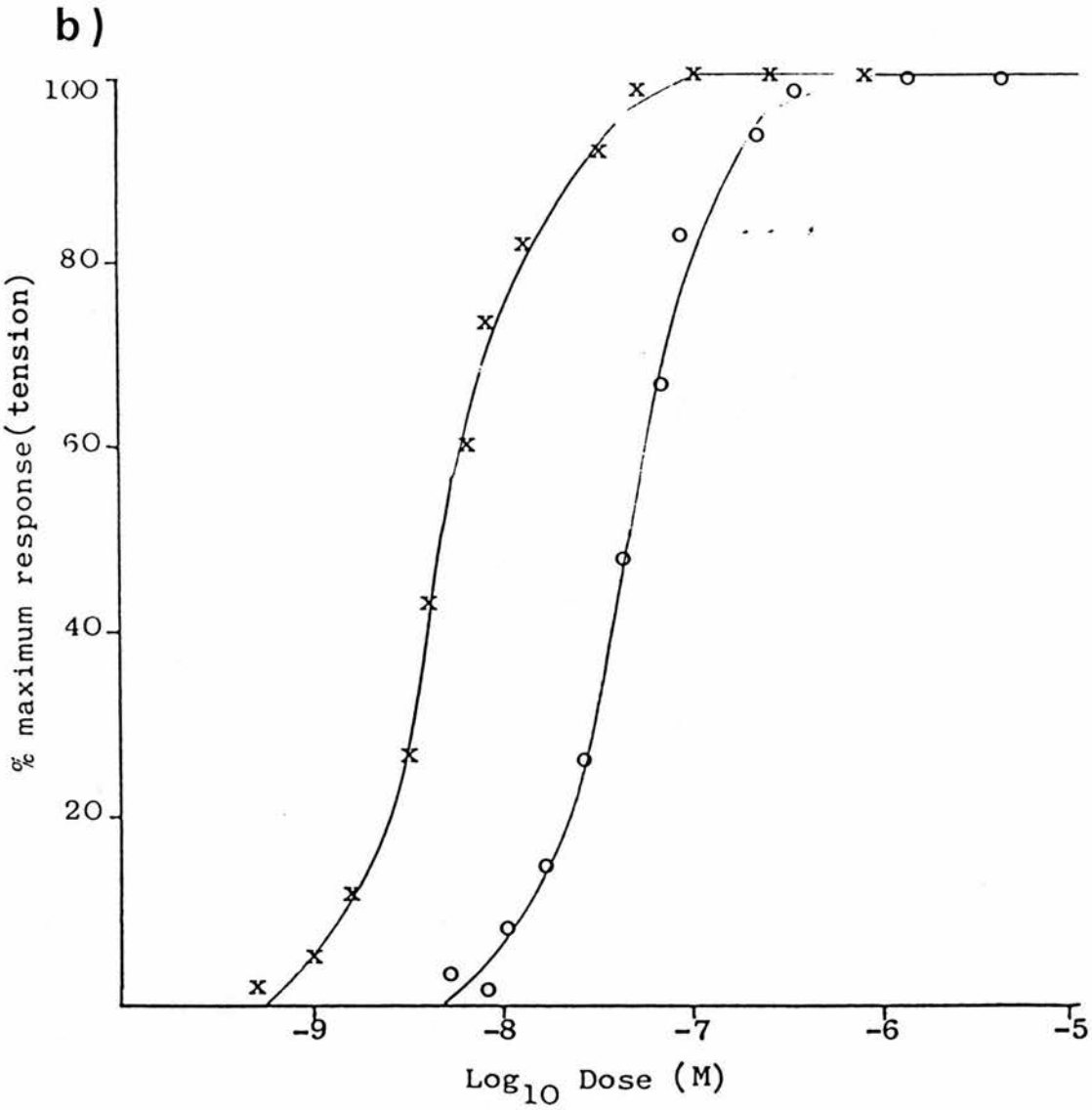
The order of potency of the three agonists used was maintained in the denervated tracheae, i.e. ISO>PROC>

FIGURE 9



Log dose - response curve produced by the addition of cumulative doses of isoprenaline (x) in the control guinea-pig trachea and its parallel shift in the presence of 2 x 10^{-6}M butoxamine (o).

FIGURE 9



Log dose - response curve showing the effect of cumulative doses of salbutamol on tension responses of the control guinea-pig trachea (x) and the parallel shift produced by the addition of 10^{-6}M ICI 118 551 (o).

Salb.

The K_a values for the series of antagonists used are listed in Table 5. The figures yield the rank potency orders of ICI 118 551 (β_2-selective) > propranolol (non-selective) > butoxamine (β_2-selective) > practolol (β_1-selective). The rank order of potency was identical when measured against Salb, indicating that both agonists are acting of the same receptor pool. The rank order of potency was also maintained when measured against both agonists in the 6-OHDA - pretreated trachea. In the cases of measuremants being made for PROC, the potencies were also in the same order. Table 5 lists the changes in K_a values of the antagonist series after 6-OHDA treatment. In all agonist cases, the β_1-selective antagonist practolol and non-selective propranolol K_a measurements significantly increased whereas the values for both of the β_2-selective antagonists significantly decreased. Levels of significance are indicated in the table.

TABLE 5

ANTAGONIST	AGONIST	CONTROL	6OHDA	
PRACTOLOL (β_1-selective)	ISOPRENALINE	5.30×10^4	1.59×10^5	ω
	SALBUTAMOL	9.46×10^4	2.83×10^5	γ
PROPRANOLOL (non-selective)	ISOPRENALINE	9.56×10^7	6.14×10^8	γ
	SALBUTAMOL	4.88×10^8	6.60×10^8	ω
BUTOXAMINE (β_2-selective)	ISOPRENALINE	6.00×10^5	2.03×10^5	γ
	SALBUTAMOL	2.48×10^6	1.49×10^6	ω
	PROCATEROL	5.68×10^5	1.33×10^5	γ
ICI 118 551 (β_2-selective)	ISOPRENALINE	4.88×10^6	3.25×10^6	ω
	SALBUTAMOL	4.96×10^7	1.11×10^7	γ
	PROCATEROL	6.30×10^6	2.05×10^6	γ

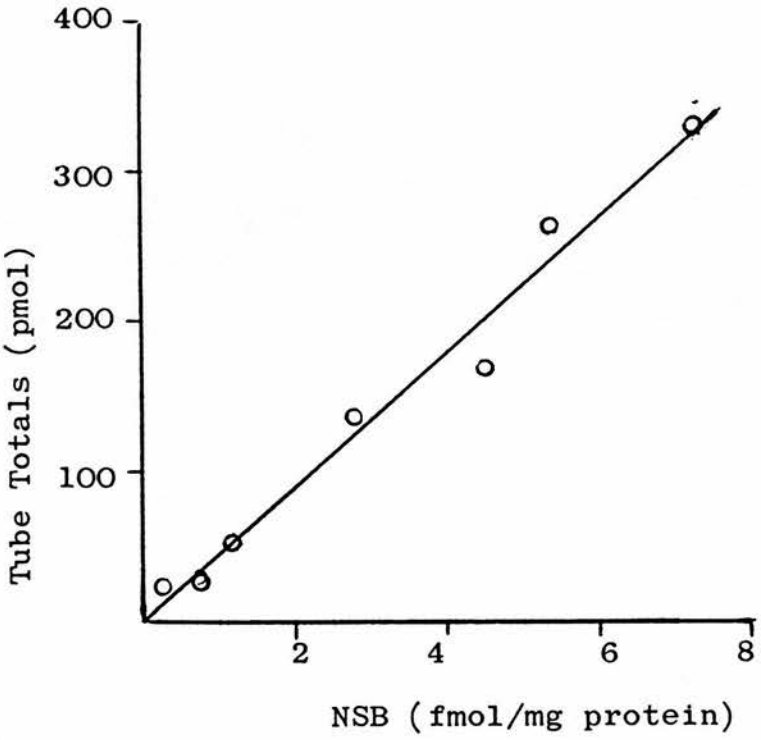
Effects of 6-OHDA pretreatment on the K_a values of a series of selective and non-selective antagonists and agonists in the guinea-pig trachea.
γ denotes significant difference from control at 1%,
ω denotes a 5% level of significance.

Binding Studies.

A series of binding studies on guinea-pig cardiac microsomal membranes were carried out to test for an agreement of data with the isolated atria experiments. For each experiment, a homogenate of 20 guinea-pig hearts was required, separated into atria and ventricles or combined. Since in the isolated organ work the adult- and neonatal-treated guinea-pigs behaved in an identical manner, adults were used for the binding studies since they provide a much greater weight of cardiac tissue per animal.

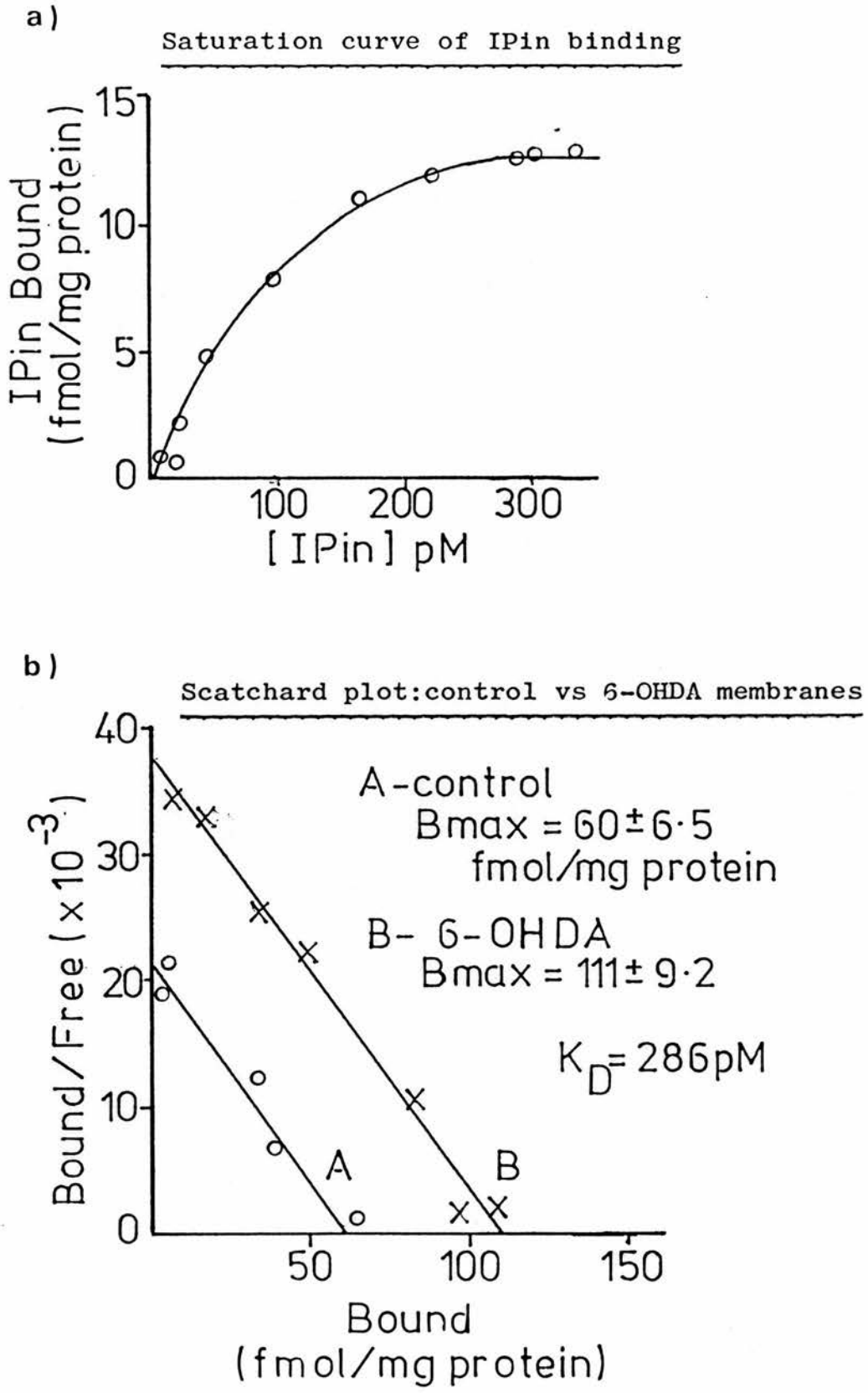
The radioligand used for the studies was (-)^{125}I-iodo-pindolol (IPin) and it was first tested to see if its binding properties conformed to those required. It was confirmed that the non-specific binding (NSB) rose linearly with total radioactivity present (see Fig 10), this linear relationship making its elimination by simple subtraction possible. NSB was linear in all control and 6-OHDA cases , any discrepancy in their respective results therefore not being due to a 'masking' of specific binding by alterations in NSB sites. A binding isotherm to IPin was produced, see Fig 11a. The curves were constructed in the presence of a 'saturating' concentration of 200pM (±) isoprenaline to take up the non-specific and non-stereospecific binding sites. The figure shows the saturable, concentration-dependent nature of the specific binding of IPin. Specific binding increased linearly with

FIGURE 10



The relationship of NSB as assessed by the presence of 200pM (±) isoprenaline and the total radioactivity present in the assay.

FIGURE 11


a)
Saturation curve of IPin binding
IPin Bound
(fmol/mg protein)
15
10
5
0
100
200
300
[IPin] pM
b)
Scatchard plot:control vs 6-OHDA membranes
Bound/Free (x10⁻³)
40
30
20
10
0
A-control
Bmax = 60±6·5
fmol/mg protein
B- 6-OHDA
Bmax = 111± 9·2
K_D = 286pM
A
B
50
100
150
Bound
(fmol/mg protein)

membrane concentration over the protein range employed in the study (50 - 200 µg protein per assay). Since IPin is a non-selective adrenoceptor antagonist, as expected it produced a monophasic Scatchard plot, see Fig 11b. A comparison of control and 6-OHDA combined atria and ventricle-membranes yielded Scatchard plots which showed up the gross differences in binding site density produced by chemical denervation. The density of ligand binding sites (Bmax) was approximately doubled in the denervated organs. Control hearts had a Bmax value of 60 ± 6.5 fmol/mg protein while the value for the denervated tissues was 111 ± 9.2 fmol/mg protein.

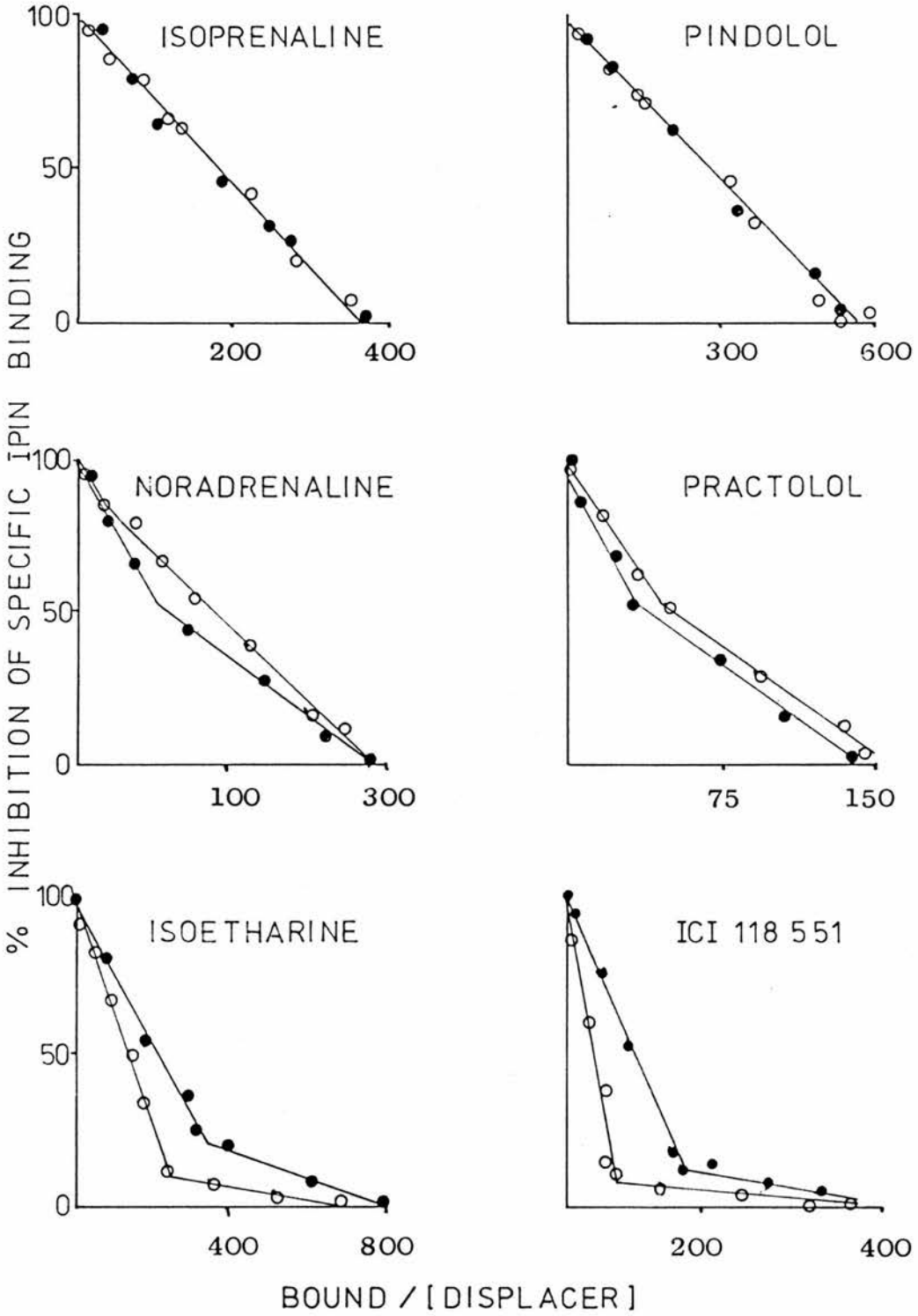
β_1- and β_2-adrenoceptor subpopulations.

The existence of two or more subpopulations of β-adrenoceptors and their relative densities was examined by means of inhibition of specific IPin binding by a series of selective and non-selective agonists and antagonists.

When isoprenaline or pindolol was used as the cold displacing ligand, the Hoffstee plots obtained were linear. This was as expected since neither compound differentiates in its binding to β_1 of β_2-adrenoceptors, see Fig 12, and this result could be explained by the presence of one or more receptor subtypes.

When the competing cold ligand was a β_1-selective agonist NA or antagonist practolol, a different type of plot emerged. The plot was now biphasic in both cases, the high affinity component (presumably β_1) being considerably larger than the low affinity (β_2) component.

FIGURE 12



Inhibition of specific IPin binding in guinea-pig atrial membranes by β_1-selective drugs noradrenaline and practolol, β_2-selective isoetharine and ICI 118 551 and non-selective isoprenaline and pindolol.
● - control, o - 6OHDA-denervated membranes.

When the β_2-selective agonist isoetharine or the β_2-selective antagonist ICI 118 551 was used, the plot also became biphasic, though in this case it was the high affinity (β_2) component which was the smaller and the low affinity (β_1) which was the larger , see Fig 12. In both control and denervated atria and ventricles, the order of potency for the agonists used was ISO > NA > isoetharine. This indicates that the major component of the adrenoceptor pool in all cases is β_1 and it further confirms the potency order of agonists as recorded from the isolated atria work mentioned earlier.

Table 6 lists the percentages of β_1- and β_2-adrenoceptors distributed in atria and ventricles of control and denervated guinea-pig hearts. The values are calculated from studies of inhibition of specific IPin binding. It may be seen that the β_1 population is predominant in both atria and ventricles but that the β_1 population is consistently higher in the ventricles. Denervation by 6-OHDA produced an increase in the atrial β_1:β_2 ratio from 69.9:30.1 to 81.0:19.0 and in the ventricles from 75.7:24.2 to 85.3:14.7. The data was assessed by computer modelling analysis in all cases.

Kinetic studies.

Kinetic experiments were carried out in order to determine the dissociation rate constant values (K_D) for the drugs when being used to displace equilibrated IPin binding on a time scale. The results obtained were analysed by computer to determine whether they

TABLE 6

Tissue	NORADRENALINE		ISOETHARINE		ICI 118 551		PRACTOLOL		MEAN	
	Control	6OHDA	Control	6OHDA	Control	6OHDA	Control	6OHDA	Control	6OHDA
Atria β1	75.3 ±5.5 (3)	90.7 ±9.0 (3)	64.3 ±4.7 (3)	73.0 ±11.2 (3)	79.3 ±9.0 (3)	85.0 ±5.0 (2)	60.5 ±17.7 (3)	73.0 ±4.3 (3)	69.9 ±10.8	81.0 ±10.5
Atria β2	24.7 ±5.5 (3)	9.3 ±9.0 (3)	35.7 ±4.7 (3)	27.0 ±11.2 (3)	20.7 ±9.0 (3)	14.0 ±5.0 (2)	39.5 ±17.7 (3)	17.0 ±4.3 (3)	30.1 ±10.8	19.0 ±10.5
Vent. β1	80.0 ±9.1 (2)	91.3 ±3.6 (3)	73.0 ±11.4 (2)	80.7 ±8.5 (3)	75.6 ±15.1 (3)	86.7 ±6.1 (3)	74.7 ±14.0 (2)	82.6 ±9.3 (3)	75.8 ±11.1	85.3 ±8.2
Vent. β2	20.0 ±9.1 (2)	8.7 ±3.6 (3)	27.0 ±11.4 (2)	19.3 ±8.5 (3)	24.4 ±15.1 (3)	13.3 ±6.1 (3)	25.3 ±14 (2)	17.3 ±9.3 (3)	24.2 ±11.1	14.7 ±8.2

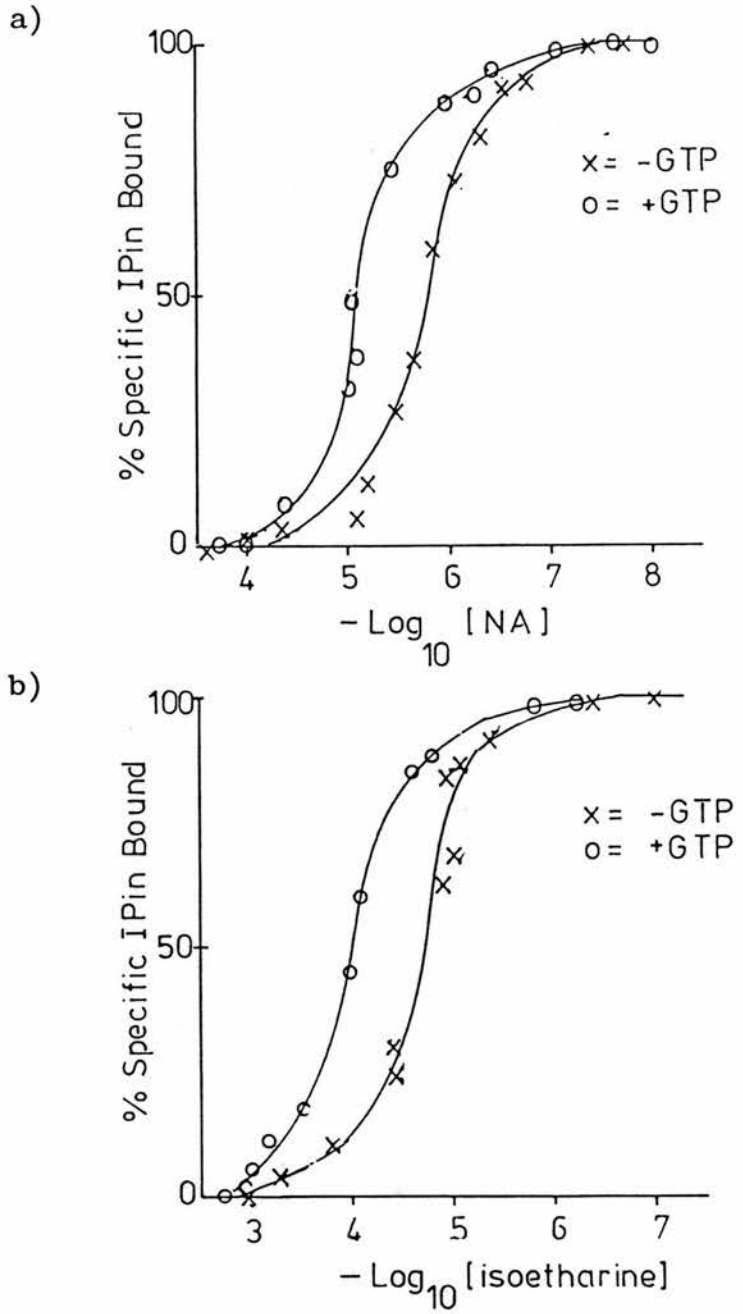
% Distribution of β1- and β2-adrenoceptors in atria and ventricles of control and 6-OHDA - denervated guinea-pigs. Number of observations shown in brackets. Results calculated by computer modelling from displacement of specific binding.

were best fitted by a one- or two-compartmental model. Non-selective ISO yielded a uniphasic displacement curve with time while selective agonists and antagonists produced biphasic displacements. K_D values were not significantly different for atria and ventricles, or between control and denervated tissues showing that the binding site affinities were unaltered by denervation. The rate constant values for β_1 and β_2 populations were as follows: NA 1.3,8.9; isoetharine 3.29,0.68; ICI 118 551 0.81, 0.31; practolol 2.5, 14.6; ISO 1.21. It seems that the rates of displacement of specific binding by selective and non-selective agonists or antagonists remains unaltered by chemical denervation.

High and low affinity states.

It has previously been shown (Kent et al,1979) that guanine nucleotides such as guanine triphosphate (GTP) which play a role in the adrenoceptor-mediated activation of the adenylate cyclase enzyme, mediate a transition of high affinity binding states of the adrenoceptor to low affinity binding states. A number of experiments were carried out to examine the ability of the nucleotide Gpp(NH)p (a stable non-hydrolysable form of GTP) to mediate this transition in the denervated guinea-pig cardiac membranes, see Fig 13. The Fig shows the alterations in displacement of specific IPin binding by β_1- and β_2-selective agonists in the presence of 10^{-4}M GTP nucleotide in the control

FIGURE 13



The effects of 10^{-4} M GTP on the inhibition of specific IPin binding by Noradrenaline (a) and Isoetharine (b) in guinea-pig ventricular membranes.

and denervated ventricular membranes. It may be seen that in both cases of control ventricles, the displacements of specific binding are shifted to the left on a $-\log_{10}$ scale indicating that the agonist was less potent in the presence of GTP, i.e. that the binding affinity was decreased. In control and 6-OHDA ventricles, the EC_{50} values for isoetharine and NA were not significantly altered (1.8×10^{-5} and 2.2×10^{-6} respectively).

In denervated membranes, the shift in the curves was of a similar order and was still observed indicating that the interconversion of high and low affinity states of the adrenoceptor still exists, and presumably it is in the same relationship as in control tissues.

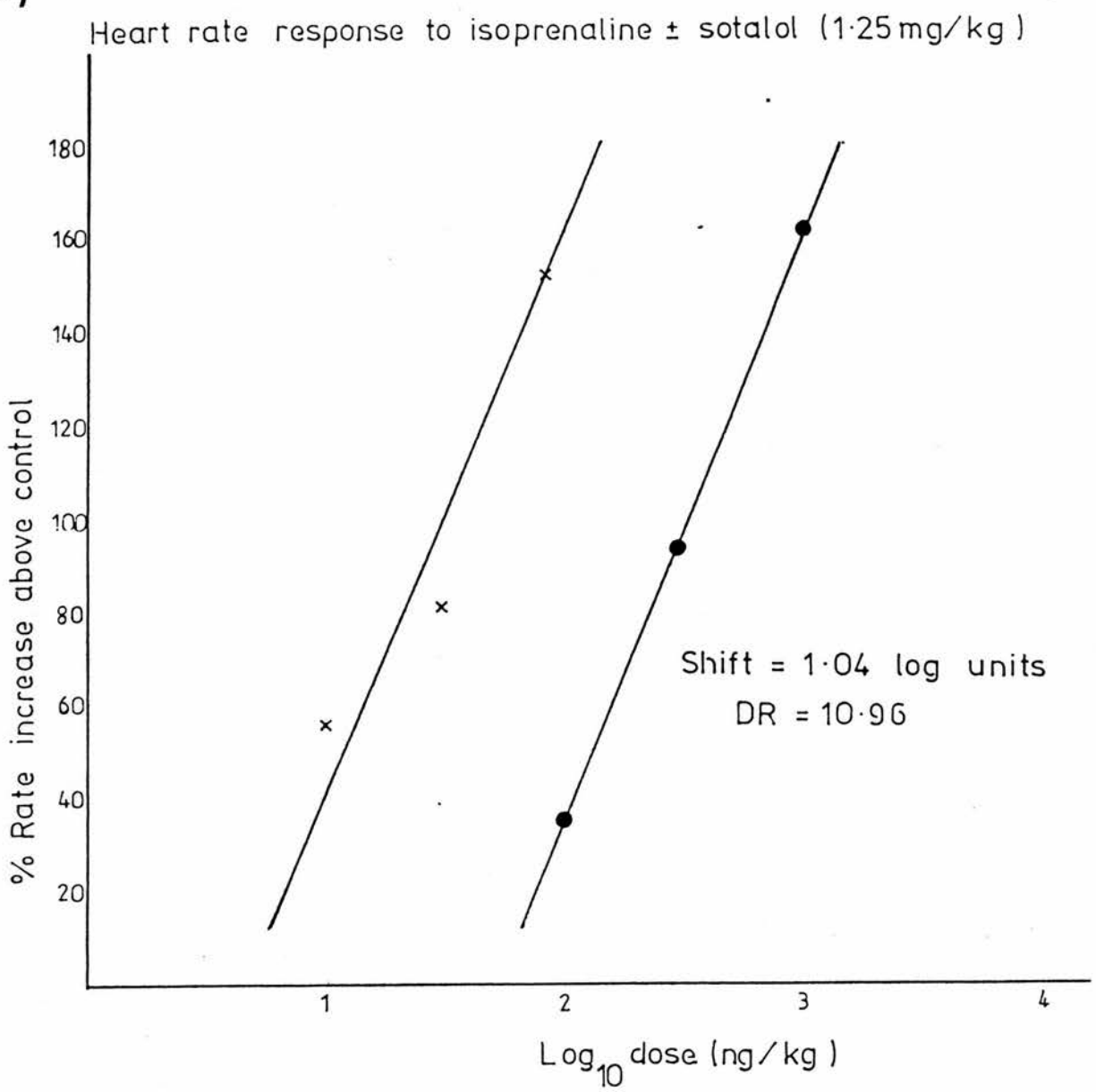
The Effects of Sotalol, Propranolol and Nadolol on the Electrocardiographs of Conscious Dogs.

The β-blocking effects of the antagonists used.

Figs 14a-c show the effects of sotalol, propranolol and nadolol on heart rate responses forinjected ISO. In all cases the log dose - response curves were shifted to the right in a parallel manner, indicating a competitive mode of action. Doses of the antagonists administered were adjusted so as to produce the same order of antagonism, since a dose of nadolol similar to that of sotalol was so potent as to cause a depression of maximum as well as a shift to the right, and we wished to examine the competitive range of antagonism only. The comparative dose ratios (DR) produced by the three β-blockers were then used to plot their effects on the ECG parameters, the DR value of propranolol being corrected by a factor of 8.65 and nadolol by 134 in order to express their results in terms of β-blocking potency alone. This method of data plotting was in order to compare their β-blocking effects with their other actions. The DR values obtained were not significantly altered by either T_3 or carbimazole treatments, nor was any change in the response to injected ISO seen. Table 7 shows the basal control levels of the measured ECG variables. There was no significant difference between the control basal levels for any of the three blockers in each condition. No significant alterations of the parameters measured were noted during the

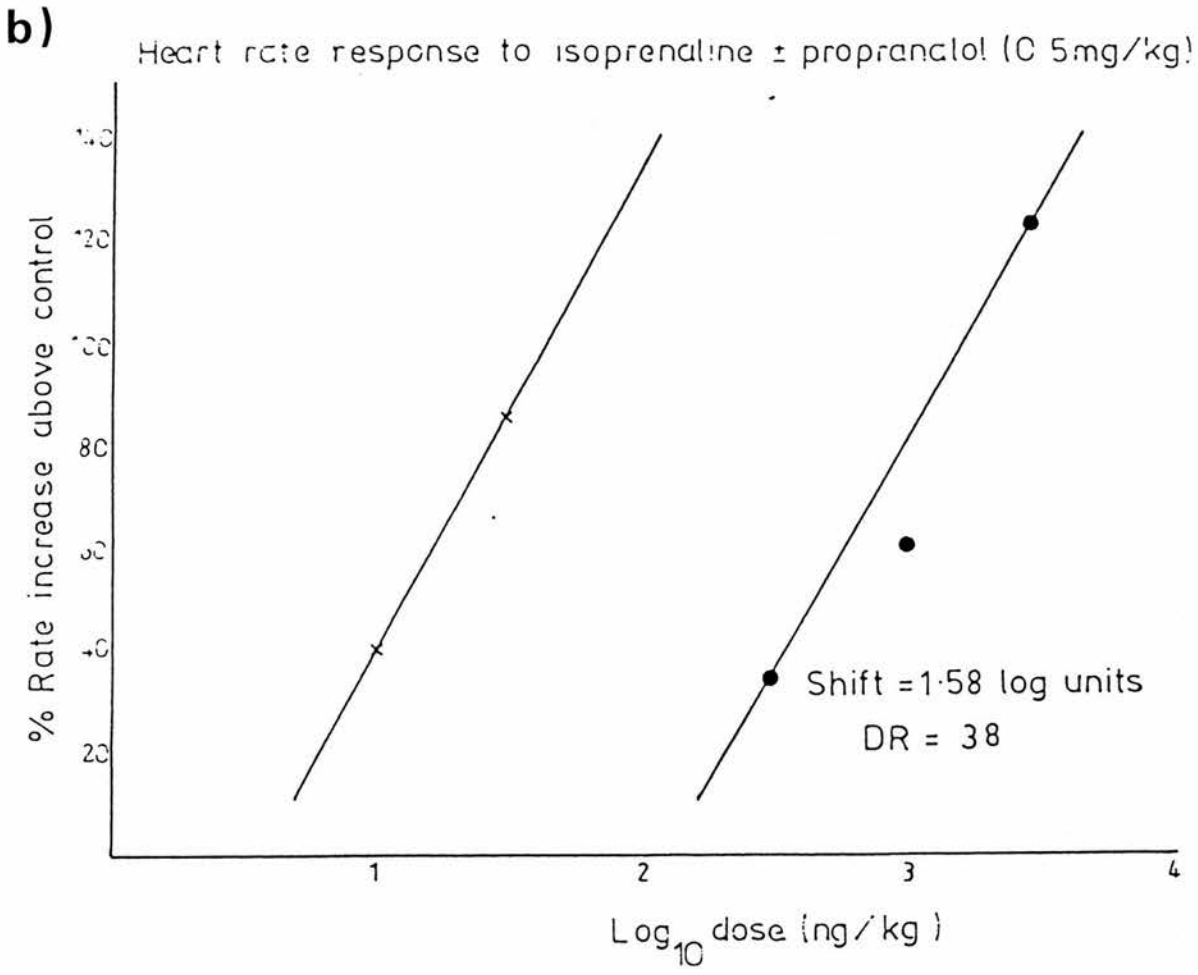
FIGURE 14

a)



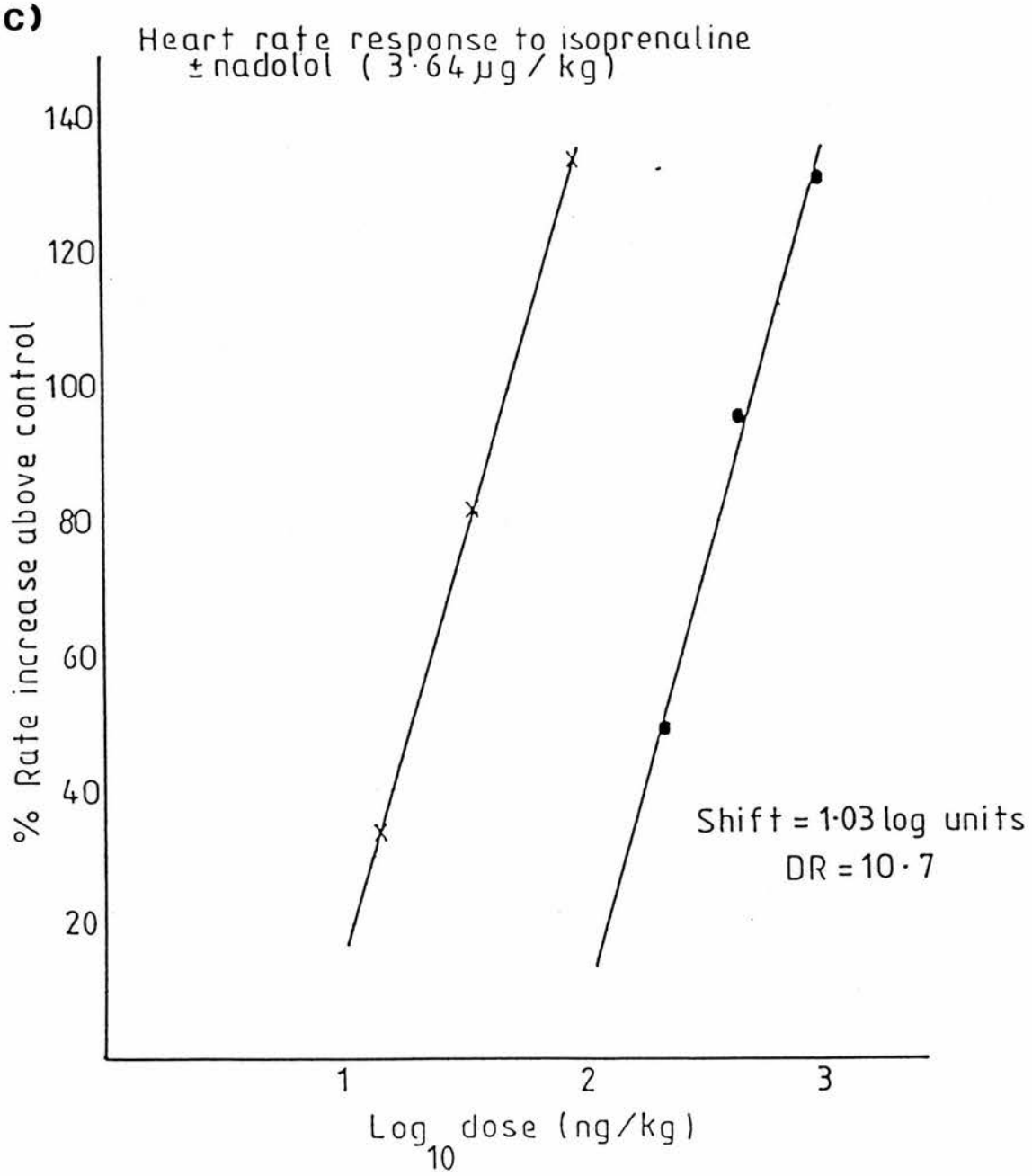
Heart rate responses to injected isoprenaline in the conscious dog before and after the administration of 1.25 mg/kg sotalol. Results shown are from duplicate estimates in each of 4 dogs.
x - isoprenaline alone • - isoprenaline in the presence of 1.25 mg/kg sotalol.

FIGURE 14



Heart rate responses to injected isoprenaline in the conscious dog before and after the administration of 0.5 mg/kg propranolol. Results shown are frem duplicate estimates in each of 4 dogs.
x - isoprenaline alone • - isoprenaline in the presence of 0.5 mg/kg propranolol.

FIGURE 14



Heart rate responses to injected isoprenaline in the conscious dog before and after the administration of 3.64µg/kg nadolol. Results shown are from duplicate estimates in each of 4 dogs.

x - isoprenaline alone • - isoprenaline in the presence of 3.64 µg/kg nadolol.

Control levels of the measured ECG variables.

	Normal Dogs			Carbimazole-treated			T3 - treated		
Variable	Sotalol (n=4)	Propranolol (n=4)	Nadolol (n=2)	Sotalol (n=4)	Propranolol (n=4)	Nadolol (n=2)	Sotalol (n=4)	Propranolol (n=4)	Nadolol (n=2)
Heart Rate (beats/min)	89±13	94±17	84±29	84±21	90±8	93±7	135±16 *	130±15 *	143±20 *
PR interval (msec)	116±9	112±4	112±1	118±6	117±6	114±1	112±3	108±6	109±1
QT interval (msec)	221±14	219±17	224±19	214±4	220±7	210±6	237±18 +	232±23 +	238±6 *
QT/RR ratio	324±7	330±38	307±81	297±74	332±37	325±15	530±68 *	521±62 *	567±93 *

The figures quoted are the means of absolute values (± S.D.) recorded prior to the administration of sotalol, propranolol or nadolol in the indicated number (n) of normal, carbimazole-treated and T3-treated conscious dogs.

* significantly different from control at 5% level
+ significantly different from control at 1% level
] Mann-Whitney rank test.

carbimazole treatment. In the T_3-treated group, however, heart rate (HR), QT interval and QT/RR ratio were all significantly greater than in the control group at the levels of significance indicated. No significant alteration in the PR interval was observed.
Table 8 lists the properties of the β-blockers employed in the present study.

The effects of T_3 or carbimazole treatment on circulating T_3 and T_4 levels.

Fig 15 shows the plasma levels of T_3 and T_4 in the control, T_3- and carbimazole-treated dogs. Levels of plasma hormones were detected by radioimmunoassay. Plasma T_4 was depressed by both treatments, but the effect was not significant. However, T_3 levels were significantly increased during T_3 administration as expected.

The effects of sotalol infusion on ECG parameters.

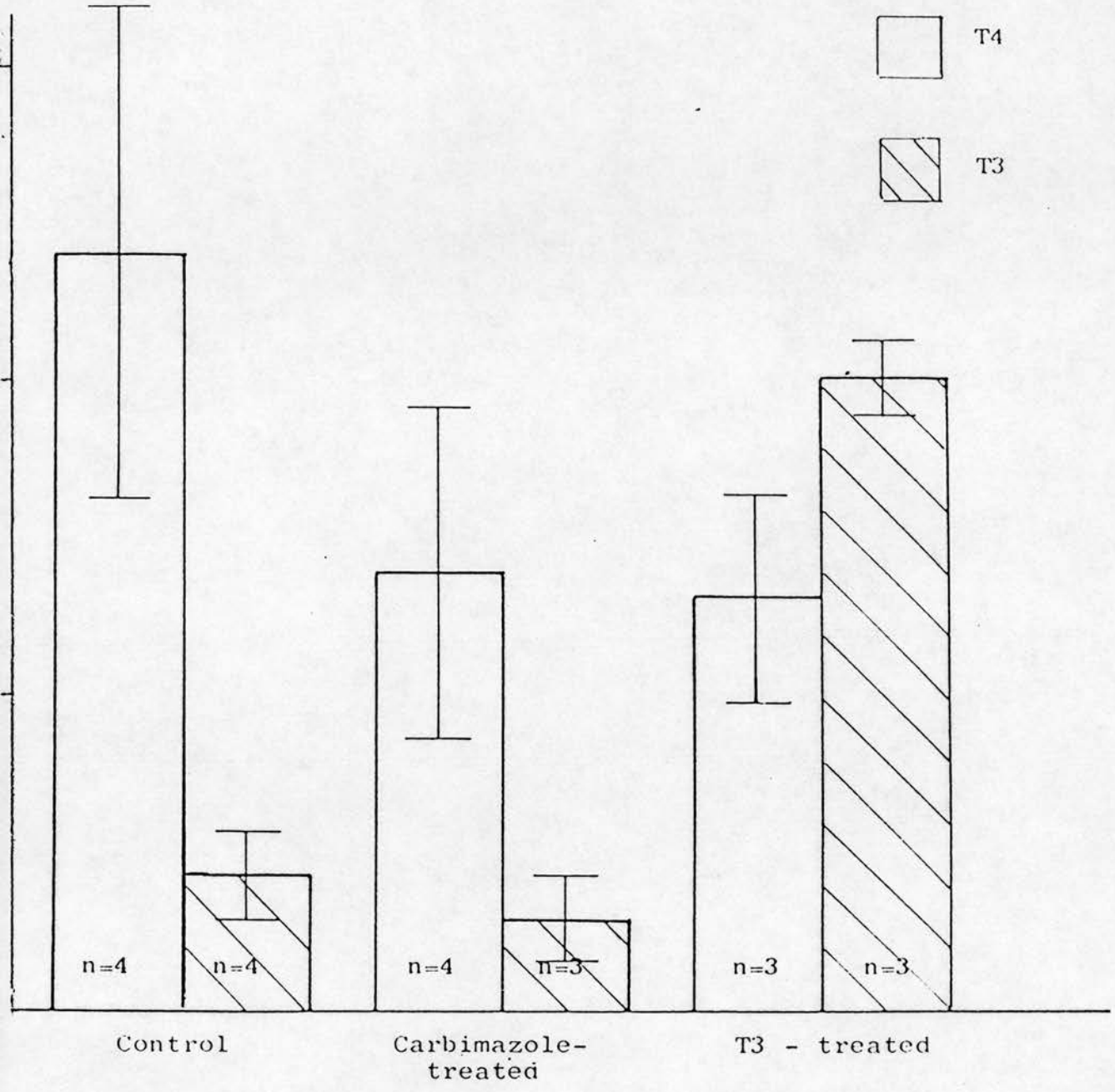
The effects of sotalol infusion on the ECG variables in control, carbimazole- and T_3-pretreated dogs are shown in Tables A(i) - (iv), B(i) - (iv) and C(i) - (iv) respectively.
Carbimazole treatment produced lethargy in the dogs, accompanied by swelling of the paws and weight increase. T_3 treatment caused a significant increase in the resting HR, with restlessness, weight loss and a decrease in body temperature.
Fig 16a shows the effects of sotalol infusion (final dose 5mg/kg) on PR interval in the control, carbimazole-

TABLE 8

Properties of the β-blockers tested

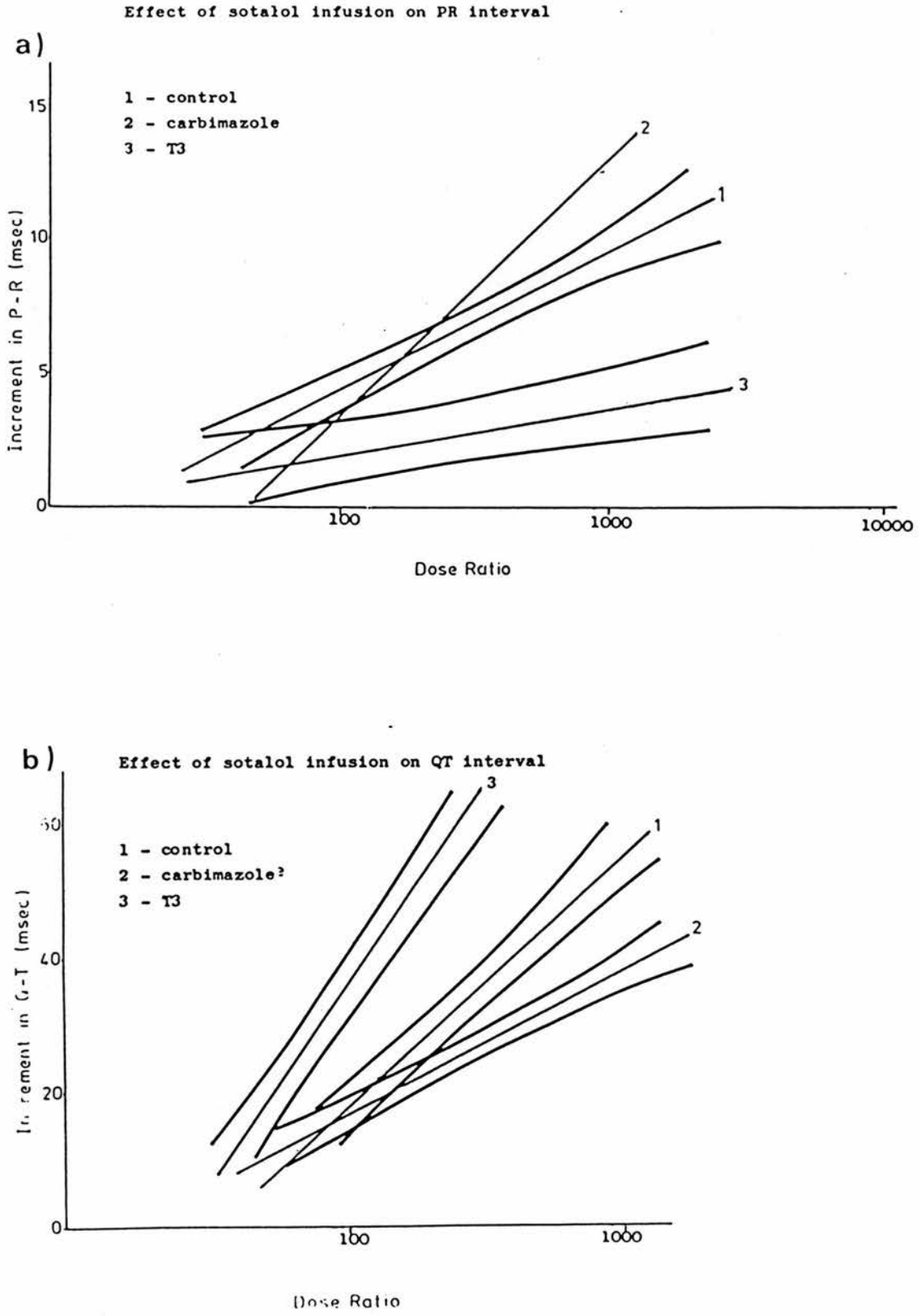
BLOCKER	CARDIO-SELECTIVITY	MEMBRANE STABILISING ACTIVITY	CLASS III ACTIVITY	INTRINSIC SYMPATHOMIMETIC ACTIVITY
PROPRANOLOL	-	++	-	-
SOTALOL	-	±	++	-
NADOLOL	-	-	-	-

FIGURE 15



Plasma levels of T4 and T3 in control, carbimazole-treated and tri-iodothyronine (T3) - treated dogs. Levels were detected by radio-immunoassay.

FIGURE 16



Sraight lines represent least squares regression fit. Curved lines represent 95% confidence limits of the regression analyses and are shown if significantly different from control.

and T_3-pretreated dogs. The PR interval increased in a dose-dependent manner with increasing sotalol concentration and, although the effect was small, it was significant. As may be seen from the confidence limits of the least squares regression analysis, the effect on the increased PR interval was significantly depressed by T_3 treatment. The effect was unaltered by carbimazole.

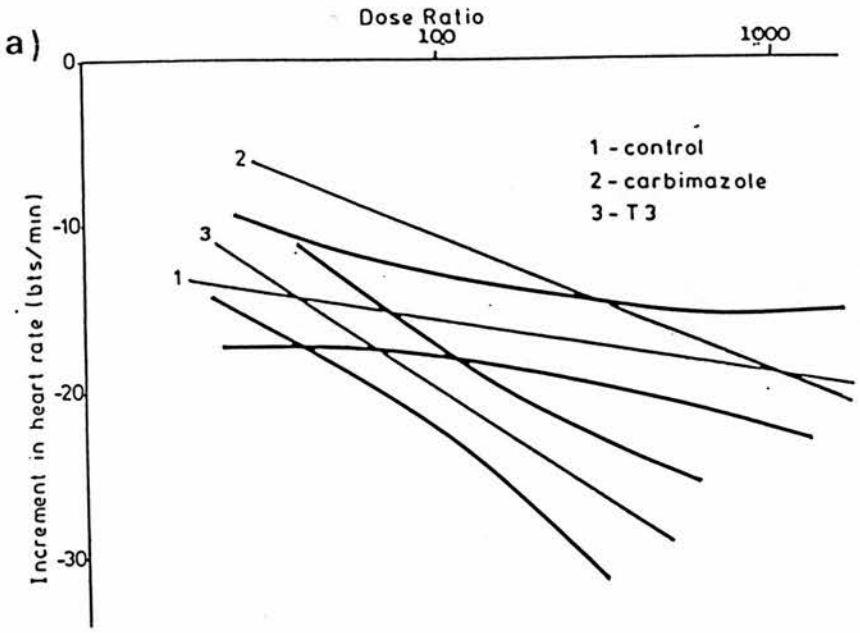
In Fig 16b the effects of sotalol infusion on QT interval are shown. In all cases there was a highly significant dose-dependent increase in QT interval with sotalol infusion. This effect was significantly depressed by carbimazole and was significantly enhanced by T_3 treatment.

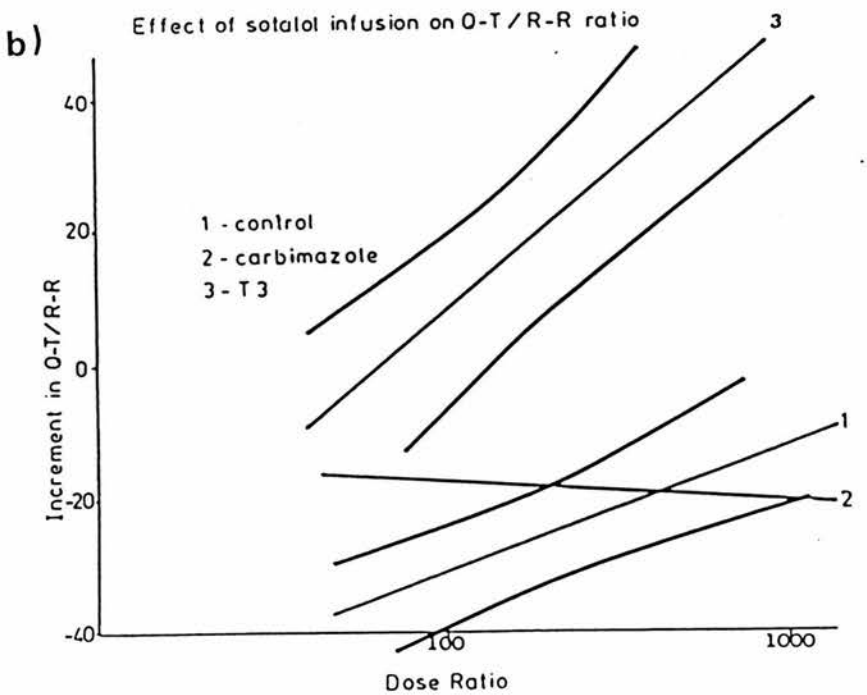
The effects of sotalol on HR are shown in Fig 17a. Although there was a consistent decrease in HR with increasing dose of sotalol, the trend was not a significant one. In the T_3 treatment, however, the decreased HR with increased dose became significant at the 5% level.

Fig 17b shows the effects of sotalol infusion on the QT/RR ratio in the three conditions. This ratio was used as an index of 'refractoriness' of the heart. The control and carbimazole states were not significantly different from each other but in T_3 treatment the ratio was significantly greater. Although the resting QT/RR ratio was higher in the T_3 group, the increments with increasing dose of sotalol remained and, indeed, control and T_3 results were parallel.

FIGURE 17

Effect of sotalol infusion on heart rate





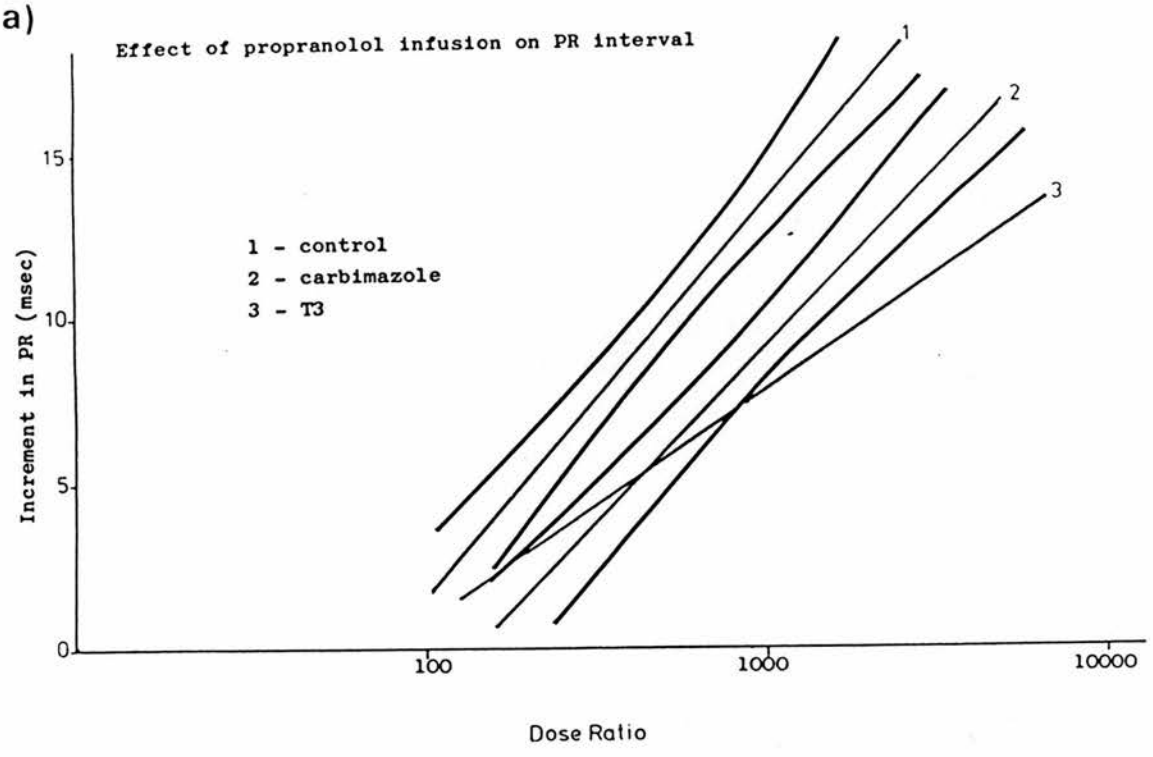
The effects of propranolol infusion on ECG parameters.
The effects of propranolol infusion on all ECG parameters in control, carbimazole- and T_3-pretreated dogs are shown in Tables D(i) - (iv), E(i) - (iv) and F(i) - (iv) respectively.

Fig 18a shows the effects of propranolol infusion (final dose 2mg/kg) on PR interval. The effects were significantly depressed by both carbimazole and T_3 treatments, both results having significant slopes at the 5% level of significance.
The effects of propranolol on QT interval are shown in Fig 18b. The slopes were all significantly different from zero at 5% level but were not significantly different from each other.

The HR effects are shown in Fig 19a. The dose-dependent decrease in HR observed in the control state was unchanged by carbimazole but was significantly depressed by T_3 treatment and the responses were shifted in a parallel manner to the right.
Propranolol infusion effects on QT/RR ratio are graphed in Fig 19b. In all cases there was a significant decrease in the ratio which was in the opposite direction from the sotalol results. Carbimazole and T_3 both failed to alter the results and all of the confidence limits overlap.

FIGURE 18

a)


Effect of propranolol infusion on PR interval
1 - control
2 - carbimazole
3 - T3
Increment in PR (msec)
0
5
10
15
100
1000
10000
Dose Ratio
1
2
3

b)

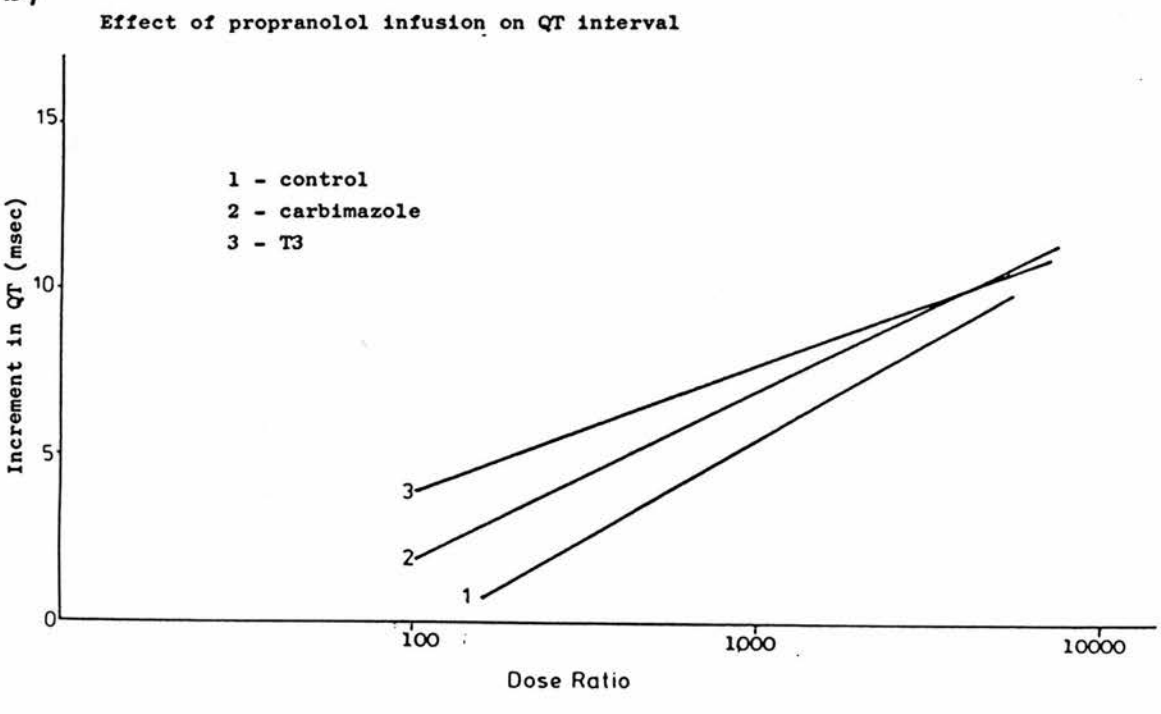
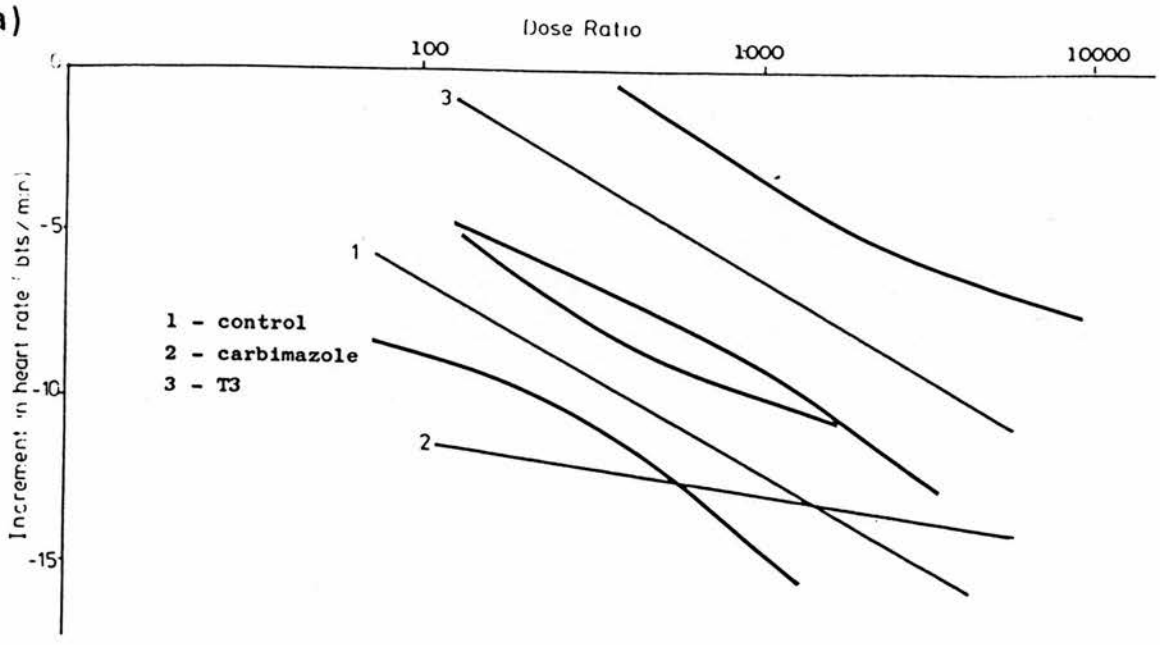
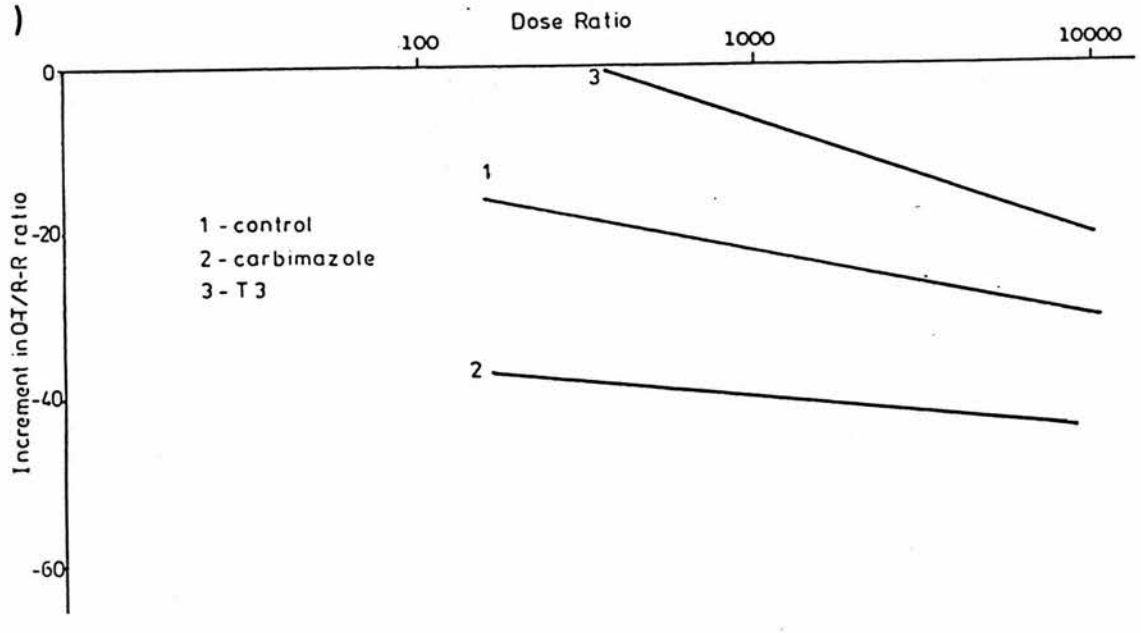

Effect of propranolol infusion on QT interval
1 - control
2 - carbimazole
3 - T3
Increment in QT (msec)
0
5
10
15
100
1000
10000
Dose Ratio
1
2
3

FIGURE 19

Effect of propranolol infusion on heart rate



Effect of propranolol infusion on Q-T/R-R ratio

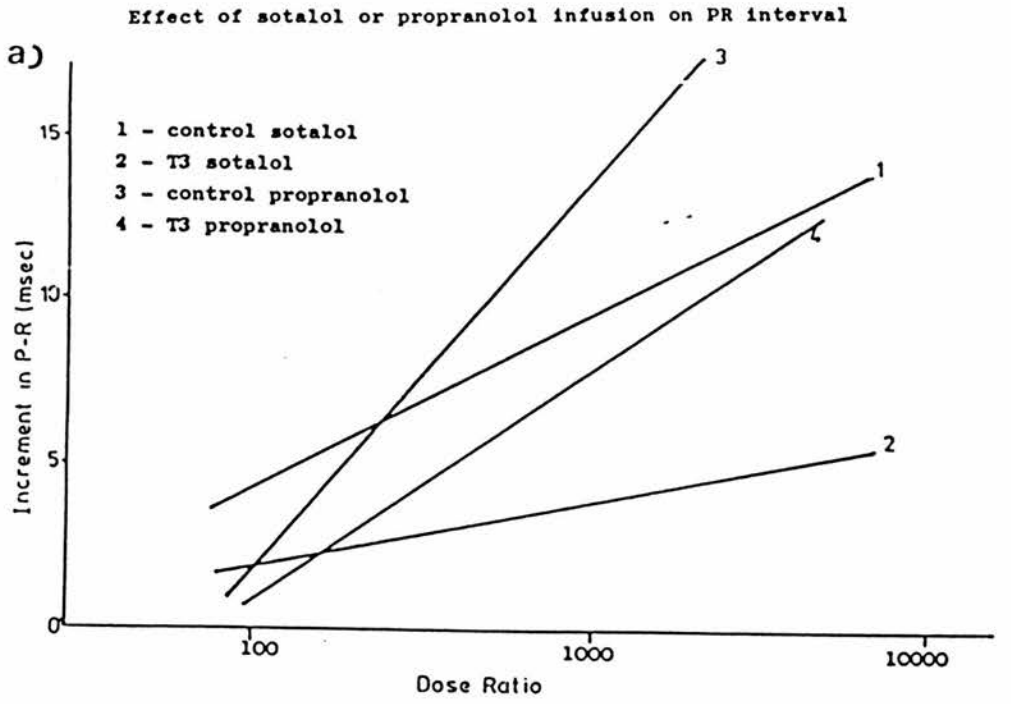


The effects of nadolol infusion on ECG parameters.

The effects of nadolol infusion (final dose 5mg/kg) are listed in Tables G(i) and (ii), H(i) and (ii) and I(i) and (ii) for control, carbimazole- and T_3-treated dogs respectively. The dose of nadolol given at the end of the infusion was the same as that for sotalol but, as was seen in Fig 14a, the β-blocking potency of nadolol is far greater then that of sotalol or propranolol and therefore the DR produced by nadolol in that concentration is 134 times greater than sotalol. Even at this range, however, nadolol had no significant effect on any of the parameters measured and so the data is not included in graph form.

Figs 20 and 21 show the combined data for sotalol and propranolol in control and T_3 states to demonstrate the selectivity of the two antagonists for the ECG variables. It can be seen that propranolol exhibits a markedly greater effect on PR interval (20a) while sotalol displays a selectivity for the QT interval (20b). Sotalol was markedly more effective in lowering HR than propranolol (21a) and this effect was enhanced by the T_3 treatment while the effects of propranolol in lowering HR were reduced. The comparison of the two antagonist infusions on QT/RR ratio shown in fig 21b show that they shift the ratio in opposite directions, sotalol increasing and propranolol decreasing the ratio, the sotalol results were parallel, the propranolol slopes not significantly altered.

FIGURE 20


Effect of sotalol or propranolol infusion on PR interval
a)
1 - control sotalol
2 - T3 sotalol
3 - control propranolol
4 - T3 propranolol
Increment in P-R (msec)
15
10
5
0
100
1000
10000
Dose Ratio
1
2
3
4

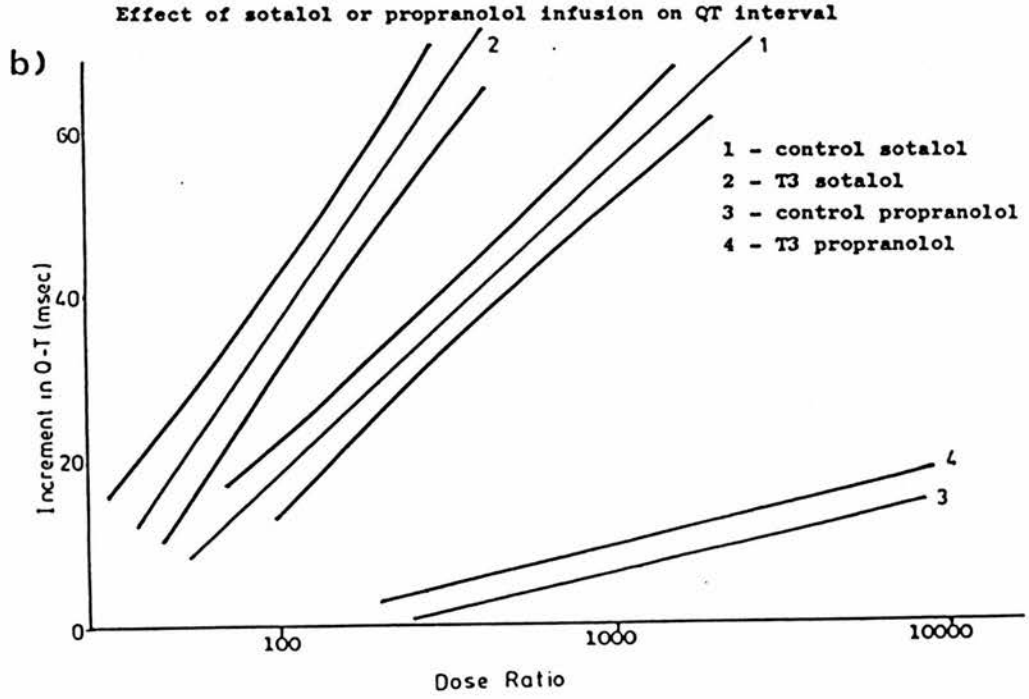
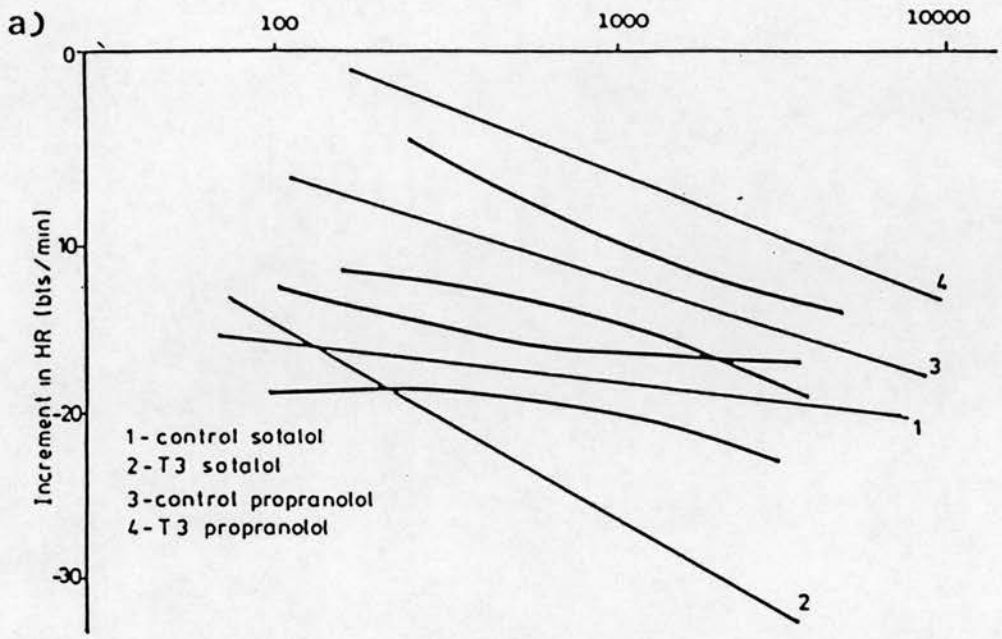
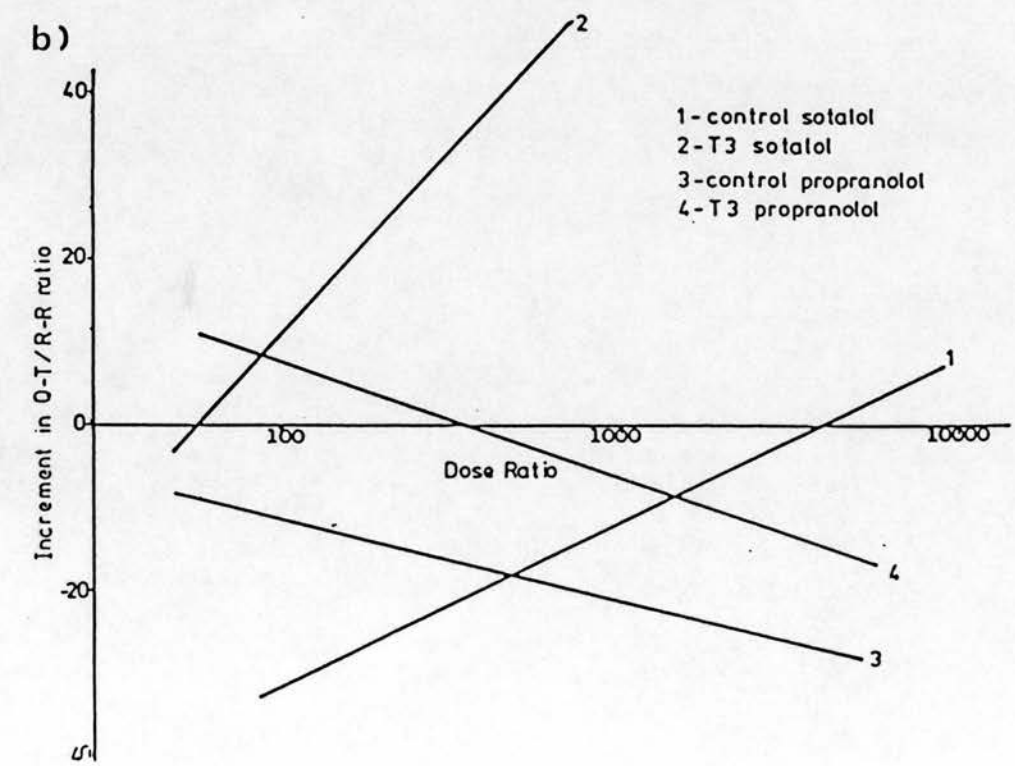

Effect of sotalol or propranolol infusion on QT interval
b)
1 - control sotalol
2 - T3 sotalol
3 - control propranolol
4 - T3 propranolol
Increment in Q-T (msec)
60
40
20
0
100
1000
10000
Dose Ratio
1
2
3
4

FIGURE 21

Effect of sotalol or propranolol infusion on heart rate



Effect of sotalol or propranolol infusion on O-T/R-R ratio



β-Blocker toxicity in the anaesthetised rat.

The β-blockers tested on conscious dogs in the previous section were then used in a series of experiments on anaesthetised rats. We examined the relative toxicity of the three antagonists sotalol, propranolol and nadolol in ventilated and unventilated rats.

Table 9 shows the numbers of cases of β-blocker poisonings reported to the National Poisonings Information Service during a 1-year period. It is interesting to note that there appears to be no correlation between the number of cases reported for each blocker and its share of the sales market. The most 'toxic' blocker appears to be propranolol with far more poisoning cases reported than expected from its relative use. Other blockers with significant usage have only a few reported poisoning cases.

The effect of sotalol or propranolol on PR interval was unaffected by ventilation. Nadolol produced no effect on PR. The effects of all three drugs on the QT intervalare shown in Fig 22. The effects were all unaltered by ventilation, the effects of nadolol being extrem ly weak. As expected, sotalol was the most potent antagonist in terms of increasing QT interval due to its Class III antidysrhythmic properties.

Fig 23 shows the relationship between the toxicities of the β-blockers when infused into ventilated and unventilated rats. In all cases, rats that were ventilated survived a much longer time and a far higher dose of

TABLE 9

β-blocker overdosage (1980 - 1981)

	MARKET SHARE (%)	NO. REPORTS (%)
PROPRANOLOL	41	81
OXPRENOLOL	21	5
ATENOLOL	18	5
METOPROLOL	6	4
SOTALOL	2	1
NADOLOL	2	0
PINDOLOL	<1	0

FIGURE 22

Effect of Sotalol, Propranolol and Nadolol on QT Interval

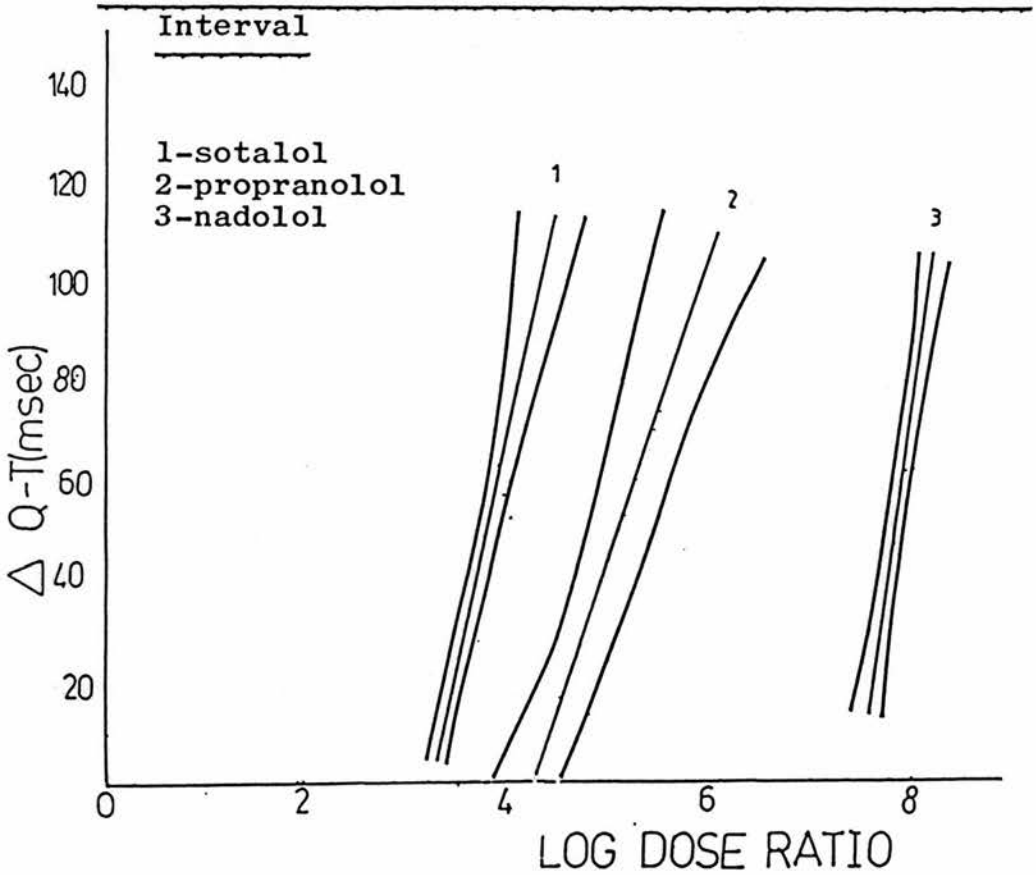
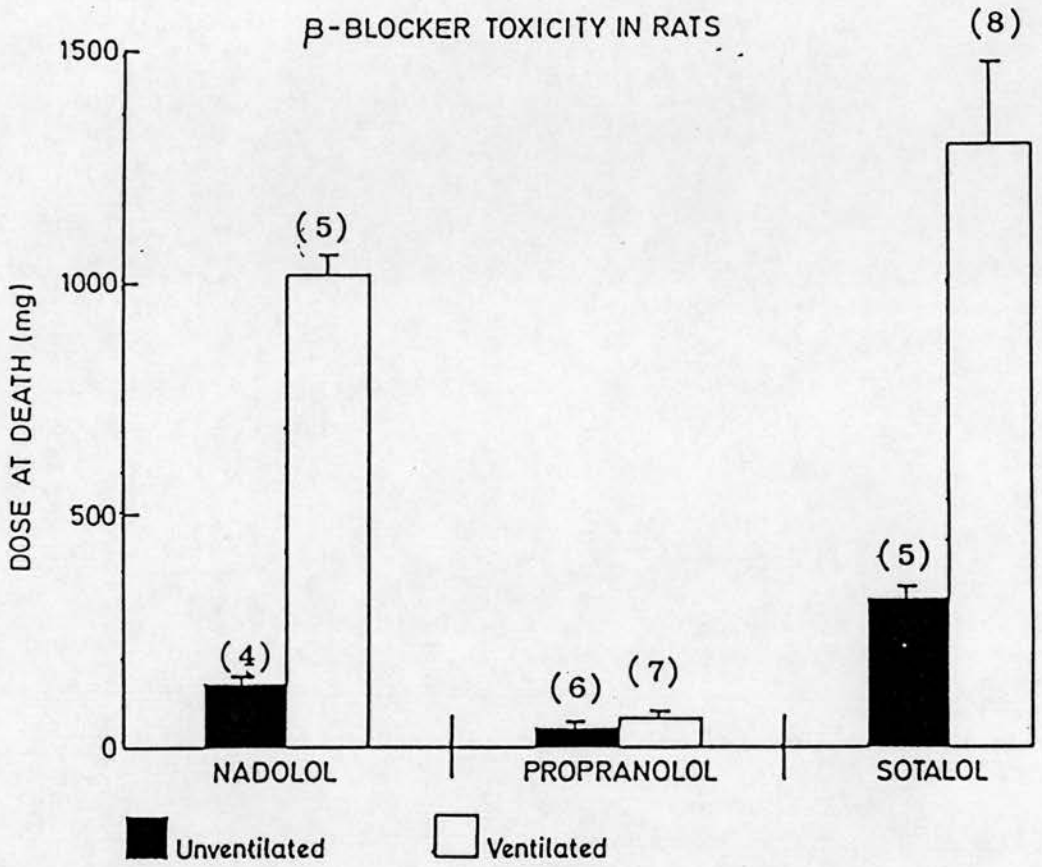


FIGURE 23

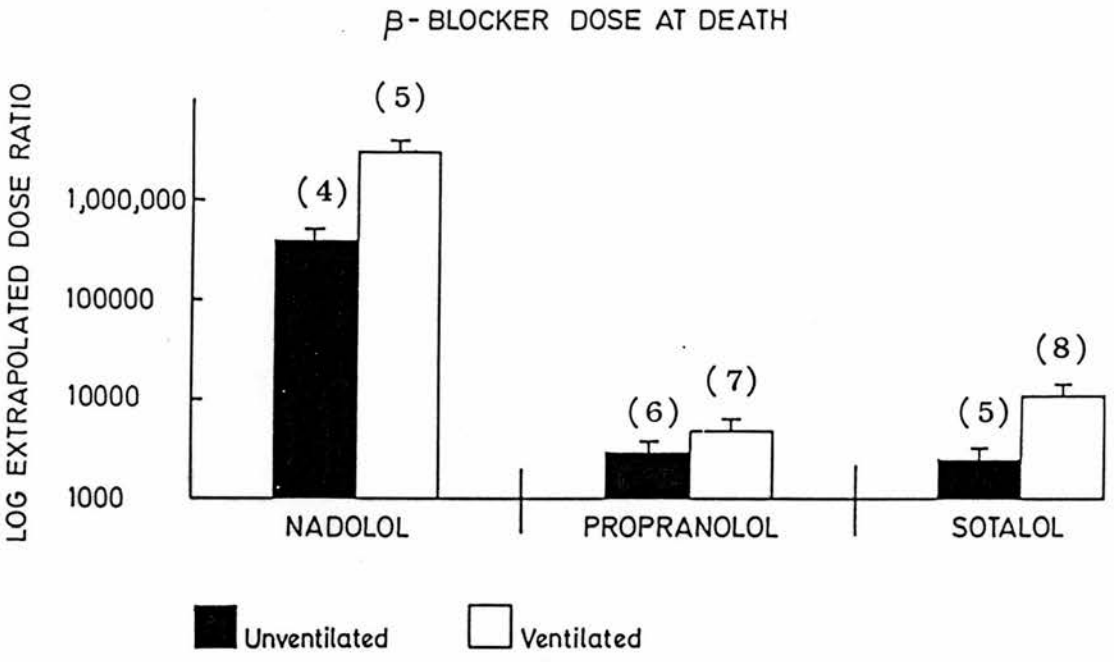


Relationship between the toxicities of β-blockers in the ventilated and unventilated anaesthetised rat. Numbers of experiments are shown in brackets.

blocker was withstood before death occurred. However, due to the very different blocking potencies of the three drugs, the results have also been expressed in terms of Dose Ratio (DR), see Fig 24. The actual DR values used in the histogram nave been extrapolated from the conscious dog data listed previously. This is because rats failed to respond to injected ISO in terms of increased HR and it was therefore impossible to produce a log dose - response curve with which to assess the DR values of the drugs directly in the rat. From Fig 24 it is apparent that nadolol, due to its far higher DR. is tolerated much better than the other two compounds and that the lethal dose is much greater in ventilated animals. The mean dose at death produced the rank potency order of propranolol > nadolol > sotalol in both ventilated and unventilated rats. The ratio of lethal doses in ventilated : unventilated rats was 1.5 for propranolol, 6 for nadolol and 4 for sotalol. However, when the data was recalculated for DR values as shown, the potency order emerged differently being propranolol >/ sotalol >> nadolol; propranolol and sotalol being approximately 130 times as toxic as nadolol.

The gross effects on ECG were then examined. Fig 25 shows sections of ECG taken during sotalol infusion in a) unventilated and b) ventilated rats. In unventilated animals, the average time of death was 28 mins. It can be seen that a gradual slowing of HR occurred with

FIGURE 24



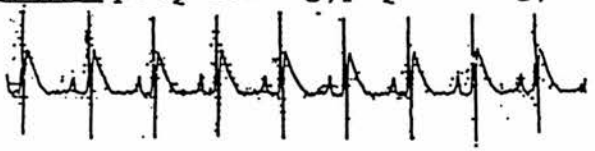
Relationship between lethal doses of β-blockers in ventilated and unventilated anaesthetised rats expressed in terms of dose ratios - figures being extrapolated from previous dog experiments. Numbers of experiments are shown in brackets.

a) ECG - SOTALOL UNVENTILATED

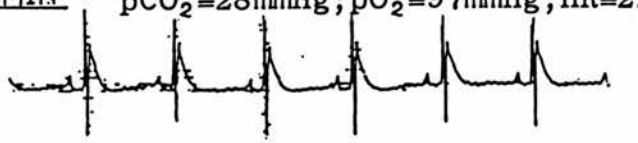
RATE OF DRUG INFUSION = 3·6 mg/min

DEAD AT 28 mins.

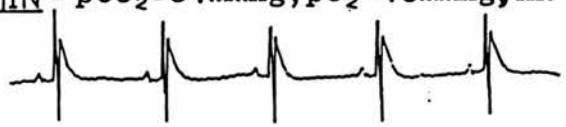
CONTROL - pCO_2 = 28 mmHg; pO_2 = 90 mmHg; HR = 307 bt/min



5 MIN - pCO_2 = 28 mmHg; pO_2 = 97 mmHg; HR = 221 bt/min



10 MIN - pCO_2 = 37 mmHg; pO_2 = 75 mmHg; HR = 185 bt/min



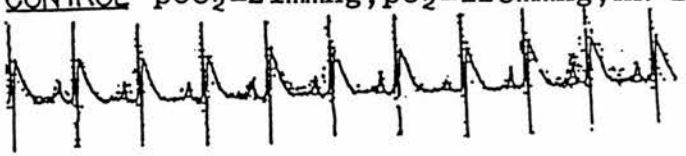
25 MIN - pCO_2 = 71 mmHg; pO_2 = 24 mmHg, HR = 108 bt/min

b) ECG - SOTALOL VENTILATED

RATE OF DRUG INFUSION = 3·6 mg/min

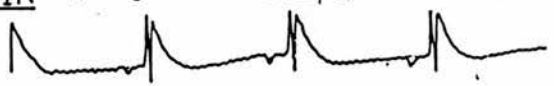
DEAD AT 138 mins.

CONTROL - pCO_2 = 21 mmHg; pO_2 = 125 mmHg; HR = 261 bt/min



10 MIN - pCO_2 = 16 mmHg; pO_2 = 141 mmHg; HR = 212 bt/min

25 MIN - pCO_2 = 10 mmHg; pO_2 = 152 mmHg; HR = 142 bt/min



115 MIN - pCO_2 = 10 mmHg; pO_2 = 150 mmHg; HR = 77 bt/min

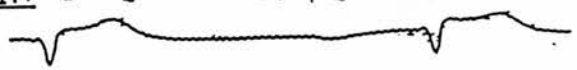


FIGURE 25

increasing dose. In control rats and also those receiving β-blocker infusions, we frequently saw a transient switch into a junctional or nodal rhythm recognised by inversion of the P waves which always reverted back to sinus rhythm. All animals appeared to be in sinus rhythm prior to the onset of cardiac death.

With nadolol (see Fig 26) the same pattern of gradual slowing with increasing dose was seen. The average time of death in the unventilated rat a) was 8 mins and in the ventilated rat b) was 85 mins. Again, sinus rhythm was followed right up until the time of death. In the case of propranolol, however, (Fig 27) a different trend was observed. An atrio-ventricular block gradually developed throughout the infusion until a full 2:1 conduction block was present a). This was repeated in the ventilated animal (b) though there was still a prolongation of survival time.

Since it was shown in Fig 23 that the DR values of the β-blockers at death were very different, it seems that death in these cases of toxicity are not due simply to β-blockade or the DR values would be expected to be more similar. Therefore the blood gas tensions were studied throughout the infusions.

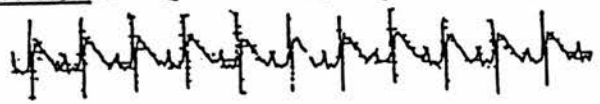
The increased survival time in ventilated rats in all three cases and the fact that pCO_2 values increased dramatically while pO_2 values decreased prior to death in all unventilated but not ventilated rats suggests that death due to β-blocker overdose is caused at least in part by respiratory depression in the unventilated rat.

a) ECG - NADOLOL UNVENTILATED

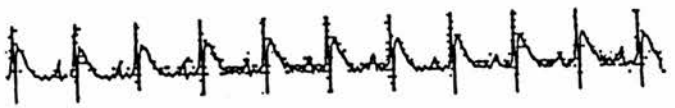
RATE OF DRUG INFUSION = 3·6mg/min

DEAD AT 10 minutes.

CONTROL: pCO_2=37mmHg; pO_2=91mmHg; HR=387bt/min



2MIN. - pCO_2=35mmHg; pO_2=85mmHg; HR=320bt/min



4MIN. - pCO_2=68mmHg; pO_2=37mmHg; HR=266bt/min

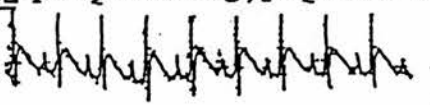
7MIN - pCO_2=94mmHg; pO_2=35mmHg; HR=64bt/min

b) ECG - NADOLOL VENTILATED

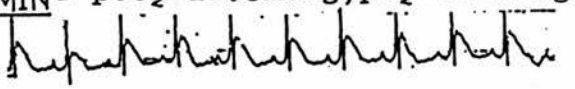
RATE OF DRUG INFUSION = 3·6mg/min

DEAD AT 92 mins.

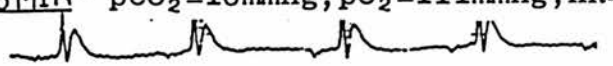
CONTROL - pCO_2=23mmHg; pO_2=72mmHg; HR=400bt/min



10 MIN - pCO_2=19.5mmHg; pO_2=102mmHg; HR=364bt/min



45MIN - pCO_2=16mmHg; pO_2=111mmHg; HR=156bt/min



80MIN - pCO_2=13mmHg; pO_2=112mmHg; HR=85bt/min

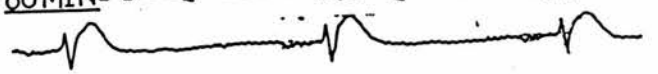


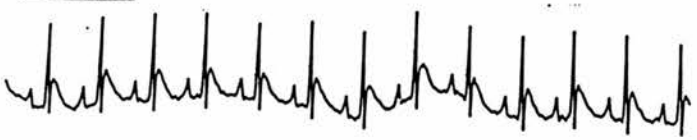
FIGURE 26

a) ECG - PROPRANOLOL UNVENTILATED

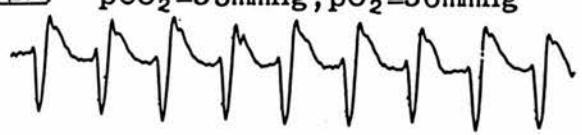
RATE OF DRUG INFUSION = 1·2 mg/min

DEAD AT 10 min. 30 secs.

CONTROL - pCO_2=36mmHg; pO_2=81mmHg; HR=381bt/min



5 MIN. - pCO_2=35mmHg; pO_2=36mmHg



6 MIN. - pCO_2=35mmHg; pO_2=34mmHg; HR=131bt/min

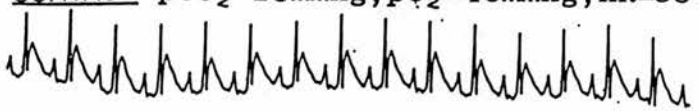
9 MIN. - pCO_2=45mmHg; pO_2=11mmHg

b) ECG - PROPRANOLOL VENTILATED

RATE OF DRUG INFUSION = 1·2 mg/min

DEAD AT 13mins.

CONTROL - pCO_2=25mmHg; pO_2=40mmHg; HR=387bt/min



5 MIN. - pCO_2=19mmHg; pO_2=42mmHg; HR=307bt/min

9 MIN. - pCO_2=9mmHg; pO_2=45mmHg; HR=176bt/min

12 MIN. - pCO_2=9mmHg; pO_2=42mmHg

FIGURE 27

Whether the respiratory depressing effect of the three antagonists is a central or peripheral effect would apparently depend on their relative pharmacokinetic properties, see Table 10. If the effects were central, it would be predicted that propranolol would be the most toxic followed by nadolol and then sotalol, since propranolol has the highest partition coefficient, all having approximately equal pKa values. However, although propranolol indeed proved to be the most toxic, nadolol was the least so and therefore the expected order was not maintained. A peripheral effect on respiration or a central effect not dependent on crossing the blood - brain barrier therefore seems a likely explanation.

E 1: THE PHARMOKINETIC PROPERTIES OF PROPRANOLOL, NADOLOL AND SOTALOL

	ABSORPTION(%)	PROTEIN BINDING (%)	PARTITION * COEFFICIENT	pK_a	HALF LIFE (hours)	METABOLISM
PROPRANOLOL	100	90	3.65	9.45	4-6	extensive
NADOLOL	15-25	25-30	0.71	9.70	12-24	minimal
SOTALOL	-	54	-0.79	9.8	5-13	minimal

*LOG PARTITION COEFFICIENT OCTANOL/WATER

TABLE 10

SECTION IV : DISCUSSION

The effects of 6-hydroxydopamine pretreatment on guinea-pig cardiac catecholamine stores.

The sympathectomy of guinea-pigs by 6-OHDA was confirmed by autofluorescence microscopy and radioenzymatic studies. The former yielded the information that there was a gross reduction in catecholamine fluorescence following 6-OHDA treatment in both atria and ventricles. The disappearance of strings of fluorescent vesicles in the denervated tissues confirms the degenerative effects of 6-OHDA on the noradrenergic nerve terminals reported by Thoenen (1972). The small amount of remaining fluorescence may be due to the small percentage of residual catecholamines or may reflect a cross-reaction with 5HT-containing granules which may have been masked in the control tissues by the dense specific fluorescence of the catecholamine-containing granules and vesicles. This would agree with Mueller et al (1969) who found that following large doses of 6-OHDA the specific fluorescence of noradrenergic and dopaminergic terminals disappeared whereas the fluorescence of 5HT terminals remained unaffected (confirmed by Malmfors and Sachs, 1968). However, the residual fluorescence is small and the SPG method was chosen because of its cross-over reactions are not as obvious as for the more popular method of Falck et al (1962) for catecholamine visualisation. The significant decrease in specific fluorescence

was confirmed by the radioenzymatic estimations of cardiac adrenaline (Ad), noradrenaline (NA) and dopamine (DA). The massive 97% reduction in Ad and NA content was considered to be evidence of cardiac sympathectomy. The approximate 10 : 1 ratio of NA : Ad observed in both control and denervated hearts indicates that the residual amines are of cardiac origin. The resistance of DA stores, seen by only a 30% reduction, confirms to some extent the findings of Kimata (1965) who found that bilateral cervical sympathectomy of the dog reduced cardiac NA markedly with no concomitant change in DA concentration. Even though we found DA content to be reduced by 1/3, it appears that DA may continue to be synthesised in cardiac muscle following denervation. The plasma catecholamines were not reduced to the same extent as cardiac catecholamines : NA was reduced by 87% and Ad by 53%, no data being available for DA. Since the adrenals are said to be completely resistant to sympathectomy with the chromaffin cells remaining intact (Mueller et al, 1969), this could be purely a result of adrenal compensation. They found that 6-OHDA treatment sufficient to almost totally destroy cardiac nerve terminals of the rat left those of the adrenal medulla intact. Concomitantly, tyrosine hydroxylase activity (the rate-determining step of NA synthesis) disappeared from the heart but greatly increased in the adrenal medulla indicating a compensatory machanism via trans-synaptic induction of the tyrosine hydroxylase enzyme was in

operation. Since this increased enzyme activity was also noted by Johnnson et al (1976) in rats depleted by guanethidine, it is likely that the same compensatory machanism occurs here. It was proposed that the increased tyrosine hydroxylase activity is mediated by an increased activity of the preganglionic nerves of the adrenals (Dahlström and Häggendal, 1973) which, as described previously, are unaffected by 6-OHDA pretreatment.

The effects of chemical denervation on the responses of guinea-pig atria to adrenergic stimuli.

Denervation by 6-OHDA is now accepted as affecting all levels of the cardiac conduction system. In 1982, Godin et al found that chemical sympathectomy of dogs with a single injection of 50mg/kg 6-OHDA caused the spontaneous cardiac cycle length to rise from 370 $\pm$ 16 msec in controls to 514 $\pm$ 15 msec in depleted dogs. This was not supported in our study at the same dose in guinea-pigs and no such alteration in heart rate (HR) was observed in the spontaneously-beating isolated atria, though the guinea-pigs were probably more efficiently denervated after a course of 6-OHDA injections. The results of Godin et al could perhaps be due to the initial uptake of 6-OHDA displacing the endogenous neurotransmitter. All studies were carried out here in the presence of phenoxybenzamine and metanephrine to block α-adrenoceptor stimulation and extra-neuronal uptake to remove any complications of these affecting β-adrenoceptor responses. Langer and Trendelenberg (1969) found that propranolol

was less effective in tissues against L-NA than L-isoprenaline (ISO) but that the antagonism was equal if uptake mechanisms were blocked. Also, as Furchgott (1972) stated, all secondary processes influencing the drug concentration at the receptor must be accounted for. In control atria, both ISO and NA were found to produce dose-dependent chrono- and inotropic responses in right and left atria respectively and both produced the same maximum response. Salbutamol (Salb) on the other hand consistently failed to produce a maximum response when measured against that of ISO and NA, evoking a 55% inotropic and a 65% chronotropic response. These submaximal responses to Salb are in agreement with previous reports (Brittain et al, 1970; Farmer et al, 1970; Hughson and Ledsome, 1975) though absolute results did vary, the latter authors finding a 65% inotropic and a 69% chronotropic response. All groups, however, agreed that the failure of Salb to attain a maximum response permitted its classification as a 'partial agonist' at the β-adrenoceptor according to the criteria of Stephenson (1956). When 6-OHDA itself was tested in vitro it was found to be a very weak agonist for both chrono- and inotropic effects, but responses were dose-dependent. This very weak agonistic property (EMR = 600) would explain the observations of Thoenen and Tranzer (1968) with ^{3}H-6-OHDA that in the cat spleen it may be released as a false transmitter. It may be seen from Fig 6 though, that 6-OHDA produced no antagonism of response to ISO

when incubated in vitro with the atria. This indicates that it does not act directly at the β-adrenoceptor level to produce its weak positive ino- and chronotropic effects otherwise it would reduce responses to ISO by β-receptor occupation. This may therefore be further evidence for its indirect actions. In the 6-OHDA depleted atria, different results were obtained. 6-OHDA addition failed to evoke any response whatever supporting the findings of Laverty et al (1965), Heusler (1971) and Kuchii and Shibata (1972). It would therefore appear that 6-OHDA acts in control guinea-pig atria as an indirectly-acting sympathomimetic amine, producing tissue responses by displacement of catecholamines from the intact neurones, hence being unable to produce effects in the denervated tissues. This difference in activity in control and denervated atria was confirmed by our findings with tyramine. It also produced a weak positive chrono- and inotropic response in a dose-dependent manner in control atria but failed entirely to do so in depleted atria. This similarity of results further indicates an indirect nature of 6-OHDA action. The absence of response to tyramine in the denervated atria also lends evidence for near-total catecholamine depletion since Crout et al (1962) found that a 50% depletion of guinea-pig cardiac NA had no effect on responses to tyramine. It was only when cardiac NA was reduced by as much as 70% that a 50% reduction in responses to tyramine were observed. Since they found that NA pretreatment restored responses to

as much as 70% without affecting cardiac NA content, they proposed a small refillable compartment (supported by Trendelenberg and Pfeffer, 1964) but could, however, be due to NA binding to non-receptor sites. The fact that we found total abolition of responses to tyramine indicates successful depletion by the above criteria and we have either a total depletion of catecholamine stores or no uptake mechanism for 6-OHDA and tyramine or a combination of the two. In denervated atria, ISO and NA still acted as full agonists, ISO being more potent for both chrono- and inotropic effects (EMR values of 1.07 and 3.26 respectively), the values not being significantly altered by denervation. However, Salb now produced no response whatever, even in doses 1000 times that producing its 'maximum' effect in control atria. When it was added to the bathing medium it was seen to act as a full antagonist against the effects of ISO and NA producing K_a values of 2.61×10^5 and 2.46×10^5 against ISO and 1.5×10^6 and 1.38×10^6 against NA for chrono- and inotropic responses respectively. This indicated that Salb may not have the straightforward partial agonist activity previously ascribed to it. In control atria, the log dose - response curves for chrono- and inotropic responses to ISO, NA and Salb were all shifted in a parallel manner to the right by both selective and non-selective antagonists indicating a first-order β-adrenoceptor interaction of all the drugs involved. Even responses to Salb were parallel, though as seen from Fig 8 the

'maximum' was depressed, indicating either that it occupies all of the β-adrenoceptors to evoke its sub-maximal response or that there is some non-specific component of action that is susceptible to β-blockade. The order of potency of the β-blockers against the three agonists was maintained in the 6-OHDA atria, except for Salb. Since β_1-selective practolol (Pract) produced the rank NA > ISO > Salb, β_2-selective ICI 118 551 produced Salb > ISO > NA and non-selective irreversible Ro-03 7894 produced ISO > NA > Salb for both chrono- and inotropic effects, it would appear that both β_1 and β_2 subtypes coexist in guinea-pig atria subserving both chrono- and inotropic responses to adrenergic agonists, otherwise a single rank potency order for all of the β-antagonists would be expected. Since the EC_{50} values for ISO and NA were unaltered by 6-OHDA pretreatment, there was no evidence of a supersensitivity to exogenous catecholamines as we had expected from the denervation supersensitivity reports in the literature. The K_a values of the highly-selective β_2 antagonist (Bilski et al, 1980; O'Donnel and Wanstall, 1980) and the irreversible non-selective antagonist Ro-03 7894 (Nicholson and Broadley, 1978) were unaltered by 6-OHDA depletion yet the values of β_1-selective Pract were increased when measured against both β_1-selective NA and non-selective ISO. It therefore appeared that the drugs in the study were acting at different subtypes (differences in rank potencies) which are altered differently by 6-OHDA (K_a

alteration by depletion). We therefore carried out a similar series of experiments on reserpinised atria to see whether we could confirm the results of other authors or whether the anomalous results were due to our handling of the preparations. We confirmed that a denervation supersensitivity occurs following reserpinisation. Responses to ISO and Salb were both enhanced after depletion but responses to NA were in fact depressed. This confirms the results of Broadley and Lumley (1977) who found that both rate and tension responses of guinea-pig atria were shifted to the left by reserpine treatment. Since they found no alteration in responses to histamine or Ca^{2+} ions the effect may be considered β-adrenoceptor selective. They also found supersensitivity to Salb but theirs was manifested by an increase in the 'maximum' produced, ours was not. Potentiation of rate responses after reserpine has been shown in perfused hearts by Westfall and Fleming (1968) but failed to show in the isolated guinea-pig atria in the hands of Crout et al (1962). Taylor et al (1974) claimed that reserpine affected only rate responses. We found both rate and tension changes confirming the results of Westfall and Fleming (1968) and McNeill and Schulze (1972) respectively. The differences in observations may be attributable to tissue trauma etc. In the case of Taylor er al, the differences may be explained by their rate of atrial pacing (60 beats/min above resting rate) which could be too high (we paced at or just above resting rate - 210 beats/min) as if pacing is too fast, energy availability

to follow the pace may become a limiting factor and reduce the inotropic response. Tyramine and 6-OHDA both failed to evoke a response in reserpinised atria indicating a large reduction in the residual amine pool as was seen in the 6-OHDA denervated atria. The effects of reserpine on the K_a values of the non-selective antagonist Ro-03 7894 were decreased against ISO and NA but unaltered against Salb indicating a decreased blocking potency after reserpine. The selective β_1 antagonist Pract K_a values were increased against ISO and NA but again unaltered against Salb. If receptor affinity is unaltered, the supersensitivity to ISO and subsensitivity to NA indicated that agonist efficacy is altered and this change may take place beyond adrenoceptor level, eg. at the second messenger stage. The anomalous data obtained from the study suggests a dual action of Salb. It may be that Salb acts on β_2-adrenoceptors prejunctionally to produce positive chrono- and inotropic effects via the released NA (an indirect action) while acting on β_1-adrenoceptors as a full antagonist. This latter action would therefore be apparent following denervation by 6-OHDA. The supersensitivity to Salb following reserpinisation may indicate insufficient depletion of the intact adrenergic neurones. The lack of response to tyramine in the reserpinised atria may indicate that too low a dose was used to release residual pools. Our conclusions on the actions of Salb are supported by Hayes et al (1982) who found that both ISO and Salb significantly increased

the fractional release of ^{3}H-NA from guinea-pig atria. The widely-held opinion that Salb is a partial agonist at cardiac adrenoceptors appears to require revaluation. Bieth (1980) found in binding studies that Salb had zero affinity for rat lung β_2-adrenoceptors and a significant affinity for rat myocardial β_1-adrenoceptors which agrees in part with our conclusions. Baker et al , 1980) studied β-adrenoceptor distribution in the canine heart and found that receptors were disposed proportionately to blood flow and the arrival of circulating catecholamines rather than to adrenergic innervaion. The relatively few adrenoceptors at the synapse were able to account for near-maximal changes in cardiac performance. We support this statement insofaras loss of innervation produced great alteration in responsiveness to adrenergic agents.

Effects of chemical denervation by 6-hydroxydopemine on responses of the guinea-pig trachea to adrenergic stimuli

We then examined the effects of 6-OHDA pretreatment on the guinea-pig trachea where the β-adrenoceptors are thought to be of the β_2-subtype. Spontaneous-tone preparations were used since in the carbachol-contracted trachea, the concentration of carbachol employed may dictate the degree of relaxation produced by β-antagonists (Buckner and Saini, 1974) and if agonists were to produce sub-maximal responses, a high tone would exaggerate this making EC_{50} values redundant. Furchgott et al (1973) found that the trachea can take up ISO and other amines

by extraneuronal uptake and therefore blockers were included in the Krebs' solution as in the atrial experiments. Salb was found to be a full agonist in the trachea, producing a maximum response equal to that of ISO and the β_2-selective agonist procaterol (Proc). The potency order for the three was ISO > Proc > Salb. When β_1-, β_2- or non-selective antagonists were employed against the agonists, they all maintained the same rank order of potency. This indicated that in the trachea the agonists and antagonists are all acting at the same receptor pool consisting probably of only one subtype. Since this rank order of potency was retained after 6-OHDA it would appear that the subtype is unaltered by chemical denervation. The order of potency for the antagonists against all agonists was propranolol > ICI 118 551 > butoxamine > practolol. This conflicts with the order obtained by Levy and Wilkenfeld (1970) who found practolol to be more potent than butoxamine. Propranolol therefore appears to be very potent when measured against β_2-selective agonists. When the K_a values were calculated for the control and denervated tracheae, after denervation the values for β_2-selective antagonists decreased while the others increased. It therefore appears that denervation does produce some alteration in tracheal responses to adrenergic agents and again innervated and non-innervated receptors seem to be present.

CONCLUSIONS:

1) Both β_1 and β_2 adrenoceptor subtypes appear to co-exist in guinea-pig atria.
2) In intact tissues, both tyramine and 6-OHDA appear to evoke positive chrono- and inotropic responses by an indirect action.
3) Reserpinisation produces atrial supersensitivity to adrenergic agonists, chemical sympathectomy by 6-OHDA does not.
4) It appears that 6-OHDA pretreatment reduces the pre-junctional β_2-adrenoceptor population.
5) Salbutamol appears to have two actions. It seems to exert its sympathomimetic effects by releasing endogenous catecholamines to produce both positive chrono- and inotropic effects while acting directly on the β_1-adrenoceptor as a full antagonist.
6) In guinea-pig tracheae, all β-adrenoceptors appear to be of the same (β_2) subtype. The β-adrenoceptors mediating relaxation responses to adrenergic agonists do not appear to be altered by denervation and therefore are probably not innervated.

Changes in cardiac β-adrenoceptors produced by 6-hydroxydopamine pretreatment assessed by radioligand binding.

To ensure that binding studies were worth pursuing, certain criteria for the binding sites had to be satisfied. In many receptor binding experiments high affinity

racemic ligands are used as tracers with differences in binding affinities of the two isomers being ignired. The resultant observed K_D is therefore actually a hybrid of $K_D(+)$ and $K_D(-)$ which can lead to a five-fold underestimate of the active isomer K_D (Burgisser et al, 1981). We used (-)^{125}I-pindolol (IPin) as our radioligand because of its high specific activity and its non-selective β-antagonism. The absence of a catechol group on pindolol increases its specificity for β-adrenoceptors since it won't bind to non-receptor binding sites that have a pronounced affinity for catecholamines and could interfere with receptor determination (Lefkowitz, 1965). A finite number of binding sites had to be established, of rather high affinity. Thus, if increasingly higher and higher concentrations of labelled ligand are added to the microsomal membrane fraction of guinea-pig hearts, label binding should be seen to increase up to the point where all of the binding sites are occupied (saturation) and this was, in fact, found to be the case - see Fig 11a. Scatchard analysis of such a hyperbolic curve yields a linear plot with negative slope as shown in part b. The term specific binding usually refers to that component of ligand binding which is displaceable by a high concentration of a cold ligand - here we used 200μM ISO - and possessing the characteristics of true receptor binding. Non-specific binding (NSB) refers to that component of ligand binding not inhibited by high concentrations of the cold competitor

and which does not meet the criteria for true receptor binding. This condition was also met as is shown in Fig 10. NSB increased linearly with total radioactivity present and was not saturable and therefore does not represent true receptor binding. NSB may include both hydrophobic and hydrophilic non-stereospecific interactions (Wolfe et al, 1977). Its linear relationship with radioligand concentrations makes it possible to eliminate it from the binding data by simple subtraction, and it was always less than 10% total binding. The Scatchard plots of control versus 6-OHDA membrane binding isotherms for IPin yield the binding site density (Bmax) value. Combined atria and ventricles were used and it may be seen that 6-OHDA pretreatment produced an approximate doubling of the ligand binding sites of the myocardial membranes. The control value of 60 $\pm$ 6.5 fmol/mg protein increased up to an average of 111 $\pm$ 9.2 fmol/mg protein. There was no alteration in receptor affinity for the ligand caused by 6-OHDA, both control and denervated tissues having an affinity constant of 286pM. This corresponds with the results of Yamada et al (1980) who found a significant increase in β-adrenergic binding sites in the rat heart following only 2 x 50mg/kg 6-OHDA treatments, with no alteration in receptor affinity. They also found that the increased receptor density was not proportionate to the degree of increased chronotropic effects that they obtained. Densities of receptor binding sites cannot therefore

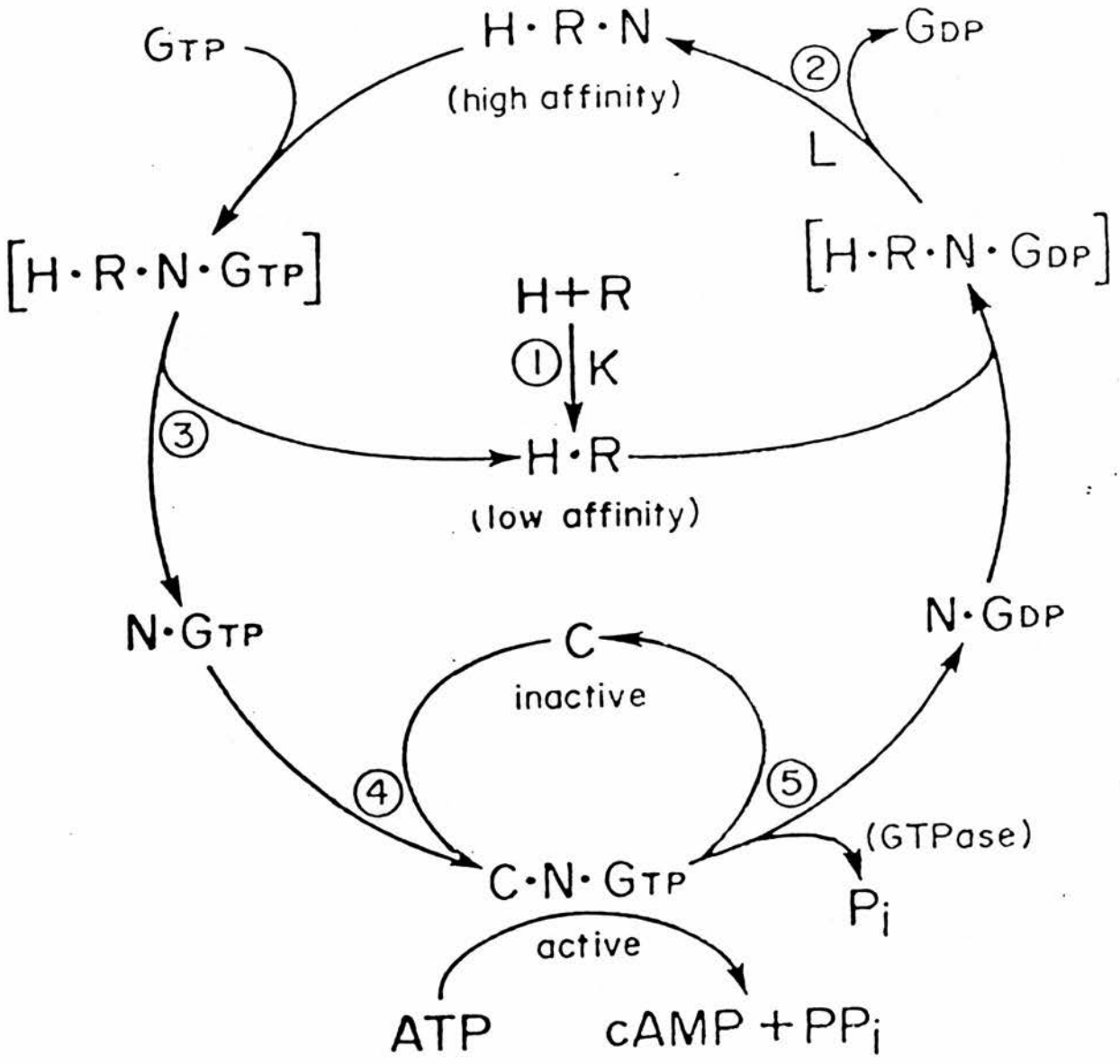
be simply equated with functional receptors. Having established initial receptor binding criteria, a series of displacement of specific binding experiments was carried out, Fig 12 shows the results of additions of a series of cold ligands to IPin incubates after equilibrium binding had been established. When the competing ligand was non-selective a uniphasic plot was observed, but when the competing ligand was selective, the resulting biphasic plot yielded information on the adrenoceptor subtypes present. Computer analysis of the data calculated the relative percentages of the two subtypes from the two components of the ligand binding plot. The iterative procedure of the computer modelling programme finds the best fit for the biphasic Hoffstee plots whether for two or more compartments and no transformation of the data is required, therefore fewer assumptions need be made and fewer errors are introduced. Each of the independent components is then assumed to obey simple Michaelis-Menten (mass action) kinetics. The size of the contribution of each component would then be due to the relative affinity of the drug for that subtype and the relative number of the subtype. Table 6 shows the calculated percentage distributions of the β_1- and β_2-subtypes and, as was expected, the major subtype present in both atria and ventricles is β_1. Following 6-OHDA pretreatment there was a significant increase in the proportion of β_1 adrenoceptors in both atria and ventricles. Since the limits of the computer modelling

technique have been placed by De Lean et al (1982) at 90% : 10% high and 10%:90% low affinity separation, perhaps in a few of the cases following denervation the results are getting very near the limits of resolution. The existence of two adrenoceptor subtypes is in agreement with the findings of Carlsson et al (1972) when they found that two adrenoceptor subtypes may coexist on the same organ mediating the same physiological response. In control atria β_1:β_2 was approximately 70:30 and in control ventricles the ratio was higher at 76:24. In the 6-OHDA atria and ventricles both ratios were significantly raised to 81:19 and 85:15 respectively and this increased β_2 population raises the possibility that the β_2 adrenoceptors identified by our study are prejunctional in origin carried on neuronal membranes in our preparation. This may explain the fact that while many authors have shown heterogeneity of β-adrenoceptors in tissues such as lung (Barnett et al, 1979) it has generally been accepted that guinea-pig ventricular β-adrenoceptors are all of the β_1-subtype (Hedberg et al, 1980). A series of kinetic experiments was carried out in the same manner to see whether denervation altered the kinetic properties of binding sites. In both sets of membranes, the 'on' reaction was too fast to measure sufficient points accurately but the 'off' reaction followed quite a long time course. Again selective agonists and antagonists produced biphasic plots and two rate constants could be calculated for each, presumably on the basis of differences

between the receptor subtypes. The rate constants remained unaltered by 6-OHDA confirming the earlier finding that receptor affinity is unaltered by 6-OHDA. Biphasic dissociation kinetics are not a unique feature of adrenoceptor binding. Similar kinetics have been described for several ligands in different receptor systems eg. the thyrotropin receptor (Verrier et al, 1974) and the acetylcholine receptor (Boyd and Cohen, 1980). It has often been stated that the intrinsic activity of the β-adrenoceptor agonist may be regarded as its ability to activate the adenylate cyclase system of a cell (Kent et al, 1979). The activation appears specific for the β-adrenoceptor since the order of potency for stimulation is ISO > Ad >NA and the response is specifically blocked by propranolol and not by phentolamine (Lefkowitz, 1977). In guinea-pig atria the ability of ISO to increase cellular cyclic AMP is related to its inotropic effects (Osnes and Øyes, 1975). While most sources agree on this for the β_1-adrenoceptor, the case for the β_2-adrenoceptor has not been quite so clearly defined. Minneman et al (1979) showed that β_2-selective agonists produced no alteration in cyclic AMP levels while evoking marked chronotropic responses. Freyss-Beguin et al (1983) correlated β_2 agonist ability to elevate heart rate with their ability to elevate cyclic AMP levels of rat heart cell cultures confirming the findings if Hazeki and Ui (1980) who showed that Salb and Proc (both β_2) both elevate cyclic AMP levels in dispersed heart cells.

However, since none of the β_1- and β_2-selective drugs are totally selective, a cyclic AMP effect may not necessarily be produced by a drug's 'preferred' receptor. Mainly though, an agonist's ability to activate the adenylate cyclase system closely correlates with the proprtion of high affinity states of the adrenoceptor formed in its presence. Mg^{2+} ions are required for the formation of the high affinity receptor-agonist complex (Hoffman and Lefkowitz, 1980) and this was included in the buffers at the appropriate concentration. Guanine nucleotides have no effects on the competition curves of antagonists and antagonist binding does not involve adenylate cyclase activation as agonist binding does. Fig 28 describes the proposed model for agonist binding. The agonist or hormone (H) binds to the receptor (R) to form a low-affinity, freely-reversible complex. This may then combine with a component of the adenylate cyclase system forming a ternary complex which is intermediate and facilitates adenylate cyclase activation by GTP. GTP then dissociates the high-affinity slowly-dissociable H-R complex reverting the adrenoceptor back to its low-affinity state and activating the adenylate cyclase system (Hoffman and Lefkowitz, 1980). The high affinity binding state of the adrenoceptor can therefore be converted to its low affinity state by the addition of GTP nucleotides to the incubate. Lefkowitz et al (1978) proposed a rank order of potency for nucleotide production of dissociation and found Gpp(NH)p > GTP > GDP > ATP = GMP.

FIGURE 28



1. Hormone binding
2. Formation of high affinity state
3. Breakdown of high affinity state
4. Enzyme activation
5. Enzyme deactivation

Kent et al (1979) found that the conversion of high affinity to low affinity binding sites by the nucleotide was dose-dependent and that at 10^{-4}M GTP all of the binding sites were in the low affinity state. We therefore used Gpp(NH)p at 10^{-4}M which is a stable non-hydrolysable GTP analogue. In control and denervated membranes alike both β_1- and β_2-selective agonist displacement curves were shifted by the addition of the nucleotide. The same order of shift was found after 6-OHDA indicating an unchanged propensity of the β-adrenoceptors to be converted to their low affinity binding states. Nucleotide linking of the receptors is apparently unaltered by denervation and it appears that the K_H/K_L ratio of the binding sites is not governed by organ innervation.

CONCLUSIONS:

1) Binding characteristics of the radioligand (-)^{125}IPin appeared to satisfy criteria that the binding sites were representative of the β-adrenoceptor.
2) Sympathectomy by 6-OHDA produces a doubling of guinea-pig heart binding sites with no alteration in affinity.
3) 6-OHDA pretreatment produced an alteration in atrial β_1:β_2 from 70:30 to 81:19 and in the ventricles from 76:24 to 85:15. It's possible that the β_2-adrenoceptors are present on neuronal membranes in the preparation.
4) The interconversion of high and low affinity states of the binding sites is unaffected by sympathectomy.

5) The data of the increased β_1 population agrees with the isolated atria data so far as Salb is concerned but not the other agonists since no supersensitivity was observed. It may therefore be that binding sites identified by radioligand binding do not directly represent a functional pharmacological or physiological receptor.

The effects of β-blockade on conscious dog ECG parameters during alteration of thyroid state.

We then went on to investigate β-adrenoceptor mediated response alteration by thyroid state. The model was the conscious dog ECG. It has long been known that a tendency towards cardiac dysrhythmias is decreased in myxoedema and that apart from the occasional extrasystole associated with a very low HR, cardiac irregularities are rare. In thyrotoxicosis, however, atrial fibrillation and other supraventricular dysrhythmias are common. In both cases the alterations in cardiac performance are largely reversible by appropriate therapy. Freedberg et al (1970) showed that atrial preparations from rabbits chemically or surgically made hypothyroid displayed electrophysiological changes in an antidysrhythmic direction. They also noted that there was no alteration in the resting potential or action potential voltages or the rate of rise of action potential but the duration of the repolarisation phase of the action potential was greatly prolonged, the converse being

demonstrated in hyperthyroidism. Prolongation of the duration of action potential and therefore a consequent increase in refractory period is the Class III anti-dysrhythmic action. Sotalol possesses this class of action and has been shown to protect urethane-anaesthetised guinea-pigs against ouabain-induced ventricular fibrillation (Singh and Vaughan Williams, 1970). In doing so it greatly prolonged the duration of action potential and the QT segment of the ECG record. There is much conflicting evidence in the literature as to whether alteration of thyroid state can affect cardiac responses to catecholamines either by a direct action of the thyroid hormones on the heart or by increasing cardiac sensitivity to catecholamines. When we shifted the log dose - response curves of HR response to ISO with the three antagonists of the study, sotalol, propranolol and nadolol all produced a shift to the right of a parallel nature. This indicates that over the dose range employed all three were acting in a competetive manner to inhibit chronotropic responses to catecholamines. The resulting dose ratio values (DR) were subsequently plotted against their effects on the dog ECG so that the results were expressed in terms of their β-blocking potencies their relative blocking effects being sotalol = 1, propranolol = 8.65 and nadolol = 134. These results agree with those of Gomoll and McKinney (1974) in the chloralose-anaesthetised dog where sotalol had 1/6 - 1/8 1f the activity of propranolol in blocking HR responses to cardio-accelerans nerve stimulation, but disagree with those of

Lee et al (1975) who found that nadolol was only five times as potent as propranolol in blocking guinea-pig HR responses to ISO *in vivo*. Since many authors have reported an increased response to catecholamines after T_4 treatment, we repeated the log dose - response curves when the dogs were made both hypo- and hyperthyroid (by carbimazole and T_3 pretreatments respectively). We found no significant changes in HR response to ISO in either of the two treatments and all three antagonists produced the same degree of shift as in controls. We therfore disagree with the findings of Hashimoto and Nakashima (1978) who demonstrated an increased response of guinea-pig atria to ISO by T_4 treatment while α-adrenoceptor mediated responses were depressed. Margolius and Gaffney (1965), however, did report similar findings to ours when they showed that T_4 or ^{125}I treatment of dogs produced no effects on arterial blood pressure or responses to endogenous or exogenous catecholamines. Teoh et al (1974) found that the increased HR of thyrotoxic patients reverted to normal following specific anti-thyroid treatment but was only partially decreased by adrenergic blockade by propranolol or by reserpine. They therefore postulated that the sinus tachycardia of thyrotoxicosis is due to combined direct effects of thyroid hormones on the heart and also to an increased sensitivity to catecholamines. This was supported by Canary et al (1957) who suggested that since adrenergic blockade only slowed the HR but did not revert it to

normal, the resultant tachycardia of thyrotoxicosis must be due to a combination of direct thyroid hormone effects and an increase of adrenergic sensitivity. Symons et al (1979) demonstrated a direct myocardial effect of thyroid hormones to produce chrono- and inotropic effects. While we found that resting HR was significantly increased by T_3 treatment, responses to catecholamines were not. Neither was there a decrease in activity after carbimazole treatment. We also found QT interval to be increased by T_3 treatment which was unexpected although Sandler (1959) also found this but it was refuted by Freedberg (1967) who reported a QT shortening. This shortening of the electromechanical systole (QT) often reported in thyrotoxicosis is mainly due to a decreased pre-ejection period, left ventricular ejection time remaining unaltered. Some authors have attributed this to a shortened isovolumetric contraction time reflecting a state of enhanced myocardial contractility (Mazzaferri and Lewis, 1978; Bough et al, 1978). Conversely, Bough et al showed that systolic time interval was proportional to the degree of severity of myxoedema and could be used as an index of the disorder. We found no significant changes in the duration of the PR interval unlike Gordan et al (1944) who showed a prolongation of PR interval in thyrotoxicosis. Our observations confirm those of Sandler (1959) and Chia et al (1971) where no alteration in the PR duration was found. THe QT/RR ratio which we used as an index of 'refractoriness' was also increased

by T_3 treatment. The hypo- and hyperthyroid states were taken as achieved when the electrophysiological and physical parameters stabilised. We measured plasma T_3 and T_4 levels during each stage to see whether there was correlation berween ECG changes and hormone levels. In control dogs, T_4 levels were high, being approximately five times higher than T_3. The differences were of the same order as those reported by Cavalieri and Pitt-Rivers (1981) who demonstrated that T_4 binding to plasma proteins has ten times the affinity for T_3, the binding being non-covalent and rapidly reversible. The principle binding proteins involved are albumin, prealbumin and thyroglobulin. T_4 depression was seen in both of the drug treatments while T_3 levels were significantly increased during T_3 treatment. Since Riddel et al (1980) found no significant alteration in the half-life of plasma propranolol in either hypo- or hyperthyroidism, we did not find it necessary to alter our programme of infusions. A 30 minute infusion of sotalol (final dose 5mg/kg) produced a significant increase in PR interval and this was also noted by Piessens et al (1974). Since this is often associated with a Class I antidysrhythmic action representing as it does an increase in conduction time from the sino-atrial node to the atrio-ventricular complex, and sotalol has only 1/300 the local anaesthetic activity of propranolol, the prolongation must be caused by some other property of sotalol. This prolongation of PR interval was significantly depressed by T_3

treatment. The effects of sotalol on QT prolongation were much more marked. THe QT prolongation effect was depressed by carbimazole treatment but enhanced by T_3. Since myxoedema is usually associated with a prolonged AP duration and thyrotoxicosis with a shortened AP duration, this alteration of sotalol potency for QT prolongation in altered thyroid state appears to be advantageous. This would especially be true in hyperthyroidism where atrial fibrillation produced by shortening of the AP duration is life-threatening. Sotalol had no significant effect in reducing resting control or carbimazole HR. Again, however, the effect was more marked in T_3 treatment. The significant trend of a dose-dependent increase in QT/RR ratio in control dogs was unaltered by carbimazole but T_3 treatment greatly increased the effects of sotalol on the ratio. Since we used the ratio as an index of cardial 'refractoriness' it again appears that the β-blocker sotalol is highly beneficial in the hyperthyroid state. When propranolol was infused over a 30 minute period (total dose 2mg/kg) a different set of trends emerged. The effects of propranolol on PR interval were more marked than those of sotalol and the effects were depressed by both T_3 and carbimazole. The effects on QT interval prolongation were very weak when compared to those of sotalol but were significant and dose-dependent., being unaltered in either thyroid state. Propranolol was less effective in reducing the resting HR than sotalol which was a result in the opposite

direction from that predicted from their relative β-blocking potencies in blocking HR responses to ISO. The HR reduction was even less obvious after T_3 and could be taken to indicate a direct hormonal action on the heart insensitive to β-blockade and hence the more effective actions of sotalol to reduce HR are probably due to its Class III activity rather than its β-blocking property. This does not agree with Howitt et al (1963) who found a significant reduction in supine HR of the thyrotoxic patient with β-blockade. The weak HR effects of both antagonists here is supported by Shanks et al (1974) who found that neither produced any effects at all on the resting HR of healthy subjects though Kofi-Ekue et al (1970) found that they were both of a similar potency in producung a significant reduction in exercise-induced tachycardia in man. Propranolol produced a reduction in the QT/RR ratio indicating a reduced refractoriness of the heart which was unaltered by either treatment. It therefore seems that propranalol would aggravate the condition of APD shortening in thyrotoxicosis and may even act to precipitate fibrillation via further reduction of the effective refractory period. Nadolol infusion over a 30 minute period (final dose 5mg/kg) produced no alterations in the ECG parameters whatsoever. This supports the observations of Lee et al (1975) who infused nadolol into conscious dogs up to 150 mg/kg and found no alterations in the ECG. Nonetheless, from its extremely high β-blocking potency against ISO we had expected some ECG

changes, especially as Vukovich et al (1976) reported that nadolol produced a reduction or remission in subjects with frequent ventricular or supraventricular ectopic beats and cardiodysrhythmias. From this β-blocking data it was shown that all three antagonists act in a competetive manner. Their effects on the ECGs are different, however, It would appear from the thyroid state data that sotalol has many advantages over the more usual drug of choice propranolol to combat the resultant dysrhythmia of thyrotoxicosis since we have shown it to be more effective in reducing HR, prolonging QT interval and increasing the effective refractory period and hence reducing the risk of reentry dysrhythmias and atrial fibrillation which occurs in 10% of patients with clinically overt thyrotoxicosis (Forfar et al, 1979). In the euthyroid patient, however, a prolongation of QT interval is not necessarily beneficial. Neuvonen et al (1981a) found that euthyroid subjects receiving 10mg/kg sotalol per day exhibited QT prolongation of up to 150 msec (35%) compared to their pre-sotalol values. Propranolol on the other hand appears to act in a detrimental fashion in the hyperthyroid state by reduction of refractory period.This though may prove to be beneficial in the hypothyroid subject and these factors of the β-blocker effects should be taken into account when deciding upon a drug of choice for controlling dysrhythmias. Neuvonen (1981a) showed that sotalol produced significant prolongation in QT interval. In patients who

took an overdose of sotalol, however, the QT interval could be up to 172% elevated (1981b) accompanied by severe tachydysrhythmias and this was concurrently reported by Kontopoulos et al (1981). A good correlation was found between the degree of prolongation, the tachydysrhythmia and the serum sotalol concentraion and in almost all cases of torsade de pointes (atypical ventricular tachycardia) an increase in QT interval preceded the tachycardia (Krikler and Curry, 1976). The QT interval can only be indicative of action potential duration (APD) if all of the ventricular fibres are firing synchronously. Therefore any condition producing asynchronous activation of the ventricular muscle fibres will produce prolongation of QT interval without affecting APD. Such conditions as in the 'long QT syndrome' are associated with ventricular dysrhythmias in contrast to a prolonged APD which, whether drug- or hypothyroidism-induced is associated with stable rhythm.

CONCLUSIONS:

1) Alteration of thyroid hormone levels produces electrophysiological changes of the canine heart.
2) Sotalol but not propranolol was effective in lowering resting HR in hyperthyroid state, possibly by Class III actions.
3) In the hyperthyroid state, sotalol is highly potent in increasing QT/RR ('refractoriness') ratio.

4) Nadolol produces no alteration of the canine ECG.

5) Sotalol would appear to be a drug of choice in cardiac dysrhythmia complicated by thyrotoxicosis.

The effects of β-blocker overdose on the anaesthetised rat

From Table 9 it was shown that there was no significant correlation between β-blocker sales and the incidence of self-poisoning. The clinical features of β-blocker intoxication reported in these cases include hypotension, bradycardia, low output cardiac failure, cardiogenic shock, respiratory depression, convulsions and coma (Khan and Muscat-Baron, 1977). It therefore seems that a large central nervous system effect is produced on overdose and effects on the central nervous system are common side-effects during β-blocker therapy. The three drugs of the previous study were therefore infused into urethane-anaesthetised rats and the ECG traces monitored until death occurred. As before both sotalol and propranolol had a significant prolongatory effect on PR interval with no change produced by nadolol. This time, however, nadolol did produce an extremely weak effect on QT interval. These effects on PR and QT intervals were unaffected by ventilation indicating a direct myocardial depressant effect. When we compared the relative fatal doses of the three drugs the apparent order of toxicity was propranolol > nadolol > sotalol. Since we had shown in the conscious dog that the DR values of

the three were very different, and since as previously explained we had extrapolated these values to the anaesthetised rat, these toxic doses are better expressed in terms of DR as shown in Fig 24. The same difficulties were obviously experienced by Imms et al (1977) in evoking rat cardiac responses to exogenous catecholamines when they stated ' it is possible that the cardiovascular system of the rat contains different adrenoceptors from those of other species and which respond differently to stimulation by catecholamines'. However, in 1979 they induced an increased HR response in the anaesthetised rat to ISO when they compared the blocking effects of practolol and propranolol on HR and peripheral resistance. Their results led them to conclude that there are both β_1 and β_2 sub-populations of adrenoceptor on the rat myocardium. Expressing our results in terms of DR produced a rather different order of toxicity with propranolol $\geq$ sotalol $>>$ nadolol, propranolol and sotalol having approximately 130 times the toxicity of nadolol. This is a much greater toxicity difference than that reported by Lee et al (1975) who found that propranolol was 20 - 30 times as depressant on the canine myocardium *in vivo*. However, the initial observation that nadolol appears highly non-toxic agrees with their later work (1978) when they introduced a 'myocardial safety index' (dose producing 50% reduction in dP/dt / dose producing 50% HR reduction) and found that nadolol was 2000 times as safe as propranolol having the least

propensity of an entire series of β-blockers to produce direct myocardial depression in the atherosclerotic rabbit. Since the drugs have very different effects on the ECG we then examined the electrophysiological changes preceding death to ascertain the mechanism by which β-blocker overdose can precipitate total cardiac failure. From the ECG traces taken during sotalol infusion (3.6 mg/min) it may be seen that although ventilation greatly prolonged the survival time of the animal, similar ECGs were produced. None of the rats exhibited dysrhythmias up until the time of death. A dose-dependent bradycardia was produced by the infusion but every P wave was followed by a QRS complex. Ventilation alone at a constant volume increases survival time by a factor of 4.3. In neither case did the gross QT prolongation induce a tachydysrhythmia. This disagrees with the observations of Neuvonen et al (1981a,1981b) who found QT prolongation in sotalol overdose was proportional to the incidence of ventricular tachydysrhythmias although they presumably found this in subjects already receiving sotalol therapy which may account for the more severe reactions. Their observations supported those of Elonen et al (1979) who also found susceptibility to ventricular extrasystole proportional to QT prolongation in sotalol overdose. The same overall pattern was observed with nadolol infusion. This time the survival time was increased by a factor of 10.6 by ventilation of the animal and in both cases no dysrhythmias

were observed up until the moment of cardiac death. Death occurred much more quickly in the case of propranolol infusion and in this case survival time was prolonged by a factor of 9.2. However, in this case an atrio-ventricular block occurred very quickly and was still present in the ventilated animals and this is probably a product of its membrane-stabilising effects. From Fig 24 it had already become apparent that death was not produced as a result of a straighforward β-blocking action or else the correction of the data for their respective DR values would have produced equal effects. The fact that ventilation alone produced such a dramatic increase in survival time raised the possibility that part of β-blocker toxicity may be via respiratory depression. When blood gas data was measured throughout the infusion periods it became obvious that in all unventilated animals blood pCO_2 rose greatly prior to death with a concomitant massive drop in pO_2. This was not observed in the ventilated experiments. It therefore appeared that in the unventilated rat all three β-blockers were acting by some mechanism to depress respiration. Mustchin et al (1965) found that a single oral dose of propranolol (80mg) in humans significantly depressed respiratory responses to CO_2 with a reduction in initial respiratory pressure. This was taken as evidence of a central depressant effect. This was supported by Campbell et al (1981) who showed a depression of respiratory response to CO_2 produced by propranolol. It could well be that the combination of hypoxia and acidaemia was a contributory and even a major factor in the cardiac arrest seen with relatively low doses of β-blockers in unventilated animals.

Perhaps in that case the three blockers in our study were producing respiratory depression by this proposed central action. It then became necessary to evaluate their pharmacokinetic properties. One of the major determinants of a drug's pharmacokinetic profile is its liposolubility. In this respect, propranolol has approximately a 400 times greater distribution coefficient than sotalol or nadolol since propranolol is lipophilic and the other two are lipophobic (Woods and Robinson, 1981). The blood - brain barrier acts as a simple lipid membrane (Mayer et al, 1959) and therefore, as expected, lipid-soluble drugs penetrate that way into the central nervous system to a much higher degree than water-soluble drugs. Propranolol has been demonstrated to be taken up centrally and brain : periphery plasma ratios of 15 : 1 have been demonstrated by Myers et al (1975) in both animal and human studies. Since only the free unionised species of the drug can cross a lipid membrane this partition coefficient becomes the most important factor in determining the distribution of the three antagonists since they all have approximately equal pKa values in the range of 9.45 - 9.8 (Turner, 1983) and in that case we would have expected a toxicity order of propranolol > nadolol > sotalol. If central respiratory depression is occurring it may be that the drugs are able to enter the ventral nervous system without having to cross the blood - brain barrier. Dev and Loeschcke (1979) demonstrated respiratory depression by

intravenous hexamethonium in the hyperventilation response to nicotine applied topically to the caudal medulla. Since hexamethonium is a quaternary ammonium compound and lipophobic, in order to produce a medullary cholinergic blockade it must have penetrated the central nervous system by another route. A central effect for all three antagonists cannot therefore be entirely ruled out. It is unlikely that respiratory depression is due to direct effects on the distal airways mediating bronchoconstriction since this was found to be negligible by Campbell et al (1981). Heistad et al (1972) and Keltz et al (1977) showed that the increased minute volume produced by adrenergic agonist infusion is blocked by propranolol but also by ventilating with 100% O_2. Adrenergic stimulation of ventilation may therefore be partly mediated via the peripheral O_2 chemoreceptors and this may be a possible site of action whereby the β-adrenoceptor antagonists can produce respiratory depression.

CONCLUSIONS:

1) Isoprenaline produced no HR response in the anaesthetised rat.
2) Neither nadolol nor sotalol produced dysrhythmias preceding death from overdose but propranolol produced a 2:1 atrio-ventricular block.
3) Ventilating the anaesthetised rat at a constant volume of air greatly increased the lethal dose of

β-blocker required.

4) Death due to β-blocker intoxication appears to be due to respiratory depression and this is reflected by the blood gases and the fact that a much higher lethal dose is required in the artificially-ventilated rat.

5) Respiratory depression by β-blockers appears to be independent of their pharmacokinetic properties and both central and peripheral respiratory depression mechanisms are possible.

SECTION V : APPENDIX

Table A.

The effects of sotalol, infused at a rate of 0.167 mg/kg/min, on HR, PR and QT intervals and QT/RR ratio in four conscious dogs. Each test was carried out in duplicate and the two test runs are shown for each dog. Recovery time of several days was allowed between tests. Where S.D. values are shown, these are calculated from a 6-beat sample of the ECG record at each time stated.

Dog 1.

Time (mins)	PR ± SD (msec)	Δ PR (msec)	QT ± SD (msec)	Δ QT (msec)	HR (bts/min)	Δ HR	QT/RR	Δ QT/RR
0	105 ± 4	-	213 ± 4	-	90	-	0.319	-
1	111 ± 4	6	217 ± 4	4	85	-5	0.307	-12
2	114 ± 11	9	227 ± 6	14	69	-21	0.261	-58
5	112 ± 6	7	231 ± 4	18	82	-8	0.316	-3
10	116 ± 6	11	239 ± 2	26	89	-1	0.354	35
15	116 ± 4	11	246 ± 3	33	77	-13	0.316	-3
20	109 ± 4	4	244 ± 4	31	78	-12	0.317	-2
25	108 ± 4	3	250 ± 3	37	71	-19	0.296	-23
30	109 ± 4	4	259 ± 2	46	68	-22	0.293	-26
0	112 ± 4	-	235 ± 5	-	70	-	0.274	-
1	112 ± 4	-	239 ± 3	4	59	-11	0.235	-39
2	113 ± 2	1	247 ± 4	12	55	-15	0.226	-48
5	114 ± 3	2	259 ± 7	24	51	-19	0.220	-54
10	114 ± 1	2	279 ± 4	44	50	-20	0.232	-42
15	113 ± 3	1	284 ± 4	49	52	-18	0.246	-28
20	112 ± 2	-	292 ± 6	57	64	-6	0.311	37
25	110 ± 3	-2	297 ± 9	62	73	3	0.361	87
30	111 ± 2	-1	307 ± 4	72	66	-4	0.338	64

Dog 2.

Time (mins)	$\overline{PR}$ ± SD (msec)	$\Delta\overline{PR}$ (msec)	$\overline{QT}$ ± SD (msec)	$\Delta\overline{QT}$ (msec)	HR (bts/min)	Δ HR	$\overline{QT}/\overline{RR}$	$\Delta\overline{QT}/\overline{RR}$
0	109	-	214±3	-	100	-	0.357	-
1	106±3	-3	210±7	-4	98	-2	0.336	-21
2	106±4	-3	214±2	-	95	-5	0.339	-18
5	110±3	1	216±6	2	88	-12	0.317	-40
10	112±4	3	225±5	11	83	-17	0.311	-46
15	115±2	6	235±6	21	77	-23	0.306	-51
20	114±3	5	244±4	30	75	-25	0.305	-52
25	116±4	7	246±5	32	76	-24	0.312	-45
30	115±4	6	247±7	33	81	-19	0.333	-24
0	109±3	-	206±3	-	96	-	0.330	-
1	112±1	3	210±3	4	88	-8	0.308	-22
2	113±2	4	212±3	6	79	-17	0.279	-51
5	118±5	9	220±3	14	89	-7	0.324	-4
10	116±3	7	229±2	23	65	-31	0.248	-82
15	115±4	6	237±4	31	76	-20	0.300	-30
20	119±1	10	241±4	35	73	-23	0.293	-37
25	120±1	11	247±3	41	74	-22	0.305	-25
30	120±5	11	244±1	38	74	-22	0.301	-25

continued.....

Table A continued.

Dog 3.

Time (mins)	$\overline{PR} \pm SD$ (msec)	$\Delta \overline{PR}$ (msec)	$\overline{QT} \pm SD$ (msec)	$\Delta \overline{QT}$ (msec)	HR (bts/min)	Δ HR	$\overline{QT}/\overline{RR}$	$\Delta \overline{QT}/\overline{RR}$
0	129 ± 7	-	223 ± 3	-	96	-	0.353	-
1	132 ± 5	3	225 ± 3	2	71	-25	0.353	-
2	126 ± 4	-3	238 ± 8	15	60	-36	0.282	-71
4	125 ± 4	-4	249 ± 5	26	68	-28	0.250	-103
6	134 ± 4	5	250 ± 4	27	73	-23	0.282	-71
10	135 ± 8	6	258 ± 5	35	76	-20	0.315	-38
16	132 ± 9	3	265 ± 6	42	72	-24	0.334	-19
26	136 ± 11	7	255 ± 9	32	62	-34	0.305	-48
34	138 ± 7	9	267 ± 20	44	62	-34	0.275	-78
38	141 ± 4	12	300 ± 8	77	71	-25	0.357	4
40	137 ± 6	8	299 ± 8	76	69	-27	0.345	-8
0	129 ± 7	-	249 ± 3	-	73	-	0.304	-
1	131 ± 8	2	250 ± 20	1	57	-16	0.236	-68
2	132 ± 5	3	244 ± 10	-5	62	-11	0.253	-51
4	135 ± 5	6	269 ± 3	20	57	-16	0.255	-49
6	138 ± 5	9	276 ± 10	27	73	-	0.335	31
8	136 ± 5	7	267 ± 20	18	51	-22	0.228	-76
10	142 ± 6	13	277 ± 20	28	50	-23	0.231	-73
12	142 ± 3	12	291 ± 6	42	55	-18	0.268	-36
14	143 ± 8	14	302 ± 3	53	50	-23	0.252	-52
16	139 ± 9	10	303 ± 4	54	55	-18	0.280	-24
18	139 ± 13	10	311 ± 1	62	63	-10	0.327	23
20	138 ± 10	9	309 ± 8	60	51	-22	0.261	-43
22	139 ± 6	10	319 ± 9	70	58	-15	0.307	3
30	141 ± 6	12	304 ± 6	55	65	-8	0.329	25
32	149 ± 10	20	313 ± 3	64	82	9	0.429	125

Dog 4.

Time (mins)	$\overline{PR} \pm \overline{SD}$ (msec)	Δ PR (msec)	$\overline{QT} \pm \overline{SD}$ (msec)	ΔQT (msec)	HR (bts/min)	ΔHR	$\overline{QT}/\overline{RR}$	$\Delta \overline{QT}/\overline{RR}$
0	116±3	-	211±4	-	108	-	0.378	-
1	114±1	-2	213±4	2	94	-14	0.334	-44
2	117±1	1	218±7	7	80	-28	0.292	-86
5	121±3	5	227±7	16	77	-31	0.293	-85
10	122±5	6	234±7	23	77	-31	0.301	-77
15	123±2	7	238±2	27	68	-40	0.268	-110
20	122±5	6	240±2	29	79	-29	0.317	-61
25	124±1	8	243±6	32	77	-31	0.313	-65
30	126±1	10	247±3	36	82	-26	0.336	-42
0	118±1	-	219±3	-	80	-	0.280	-
1	121±5	3	225±5	6	85	5	0.318	38
2	123±1	5	228±5	9	77	-3	0.293	13
5	126±5	8	236±5	17	67	-13	0.264	-16
12	128±6	10	251±2	32	67	-13	0.279	-1
15	132±1	14	257±4	38	78	-2	0.336	56
20	127±5	9	260±6	41	76	-4	0.330	50
25	132±8	14	262±3	43	68	-12	0.298	18
30	121±1	3	263±3	44	60	-20	0.261	-19

Table B.

The effects of sotalol, infused at a rate of 0.167 mg/kg/min, on HR, PR and QT intervals and QT/RR ratios in four conscious dogs pretreated with carbimazole.

Where S.D. values are shown, these are calculated from a 6-beat sample of the ECG record taken at the time stated.

Dog 1.

Time (mins)	$\overline{PR} \pm SD$ (msec)	$\Delta \overline{PR}$ (msec)	$\overline{QT} \pm SD$ (msec)	$\Delta \overline{QT}$ (msec)	HR (bts/min)	ΔHR	$\overline{QT}/\overline{RR}$	$\Delta \overline{QT}/\overline{RR}$
0	118±3	-	213±4	-	96	-	0.342	-
1	121±3	3	218±2	5	87	-9	0.316	-26
2	120±6	2	220±6	7	66	-30	0.241	-101
5	130±1	12	224±7	11	71	-25	0.266	-76
10	129±4	11	238±4	25	62	-34	0.254	-88
15	132±5	14	244±3	31	53	-43	0.217	-125
20	131±4	13	244±3	31	77	-19	0.311	-31
25	130±3	12	248±4	35	52	-44	0.215	-127
30	132±5	14	244±4	31	72	-24	0.295	-47

Dog 2.

Time (mins)	$\overline{PR} \pm SD$ (msec)	ΔPR (msec)	$\overline{QT} \pm SD$ (msec)	ΔQT (msec)	HR (bts/min)	ΔHR	$\overline{QT}/\overline{RR}$	$\Delta \overline{QT}/\overline{RR}$
0	126±2	-	218±2	-	53	-	0.187	-
1	122±3	-4	220±13	2	60	7	0.218	31
2	121±4	-5	227±5	9	53	-	0.203	16
5	141±10	15	235±5	17	46	-7	0.179	-8
10	145±10	19	240±6	22	37	-16	0.148	-39
15	150±6	24	249±4	31	47	-6	0.195	8
20	146±12	20	254±6	36	51	-2	0.218	31
25	140±5	14	253±6	35	48	-5	0.204	17
30	140±5	14	247±4	29	63	10	0.260	73

continued....

Table B continued.

Dog 3.

Time (mins)	$\overline{PR}$ ±SD (msec)	$\Delta\overline{PR}$ (msec)	$\overline{QT}$ ±SD (msec)	$\Delta\overline{QT}$ (msec)	HR (bt/min)	ΔHR	$\overline{QT}/\overline{RR}$	$\Delta\ \overline{QT}/\overline{RR}$
0	111 ± 4	-	209±1	-	98	-	0.341	-
1	111±4	-	209±4	-	104	6	0.362	21
2	111±2	-	215±9	6	114	6	0.373	43
5	115±4	4	222±3	13	89	-9	0.329	-12
10	112±4	1	230±2	21	90	-8	0.345	4
15	114±3	3	240±3	31	88	-10	0.352	11
20	114±3	3	244±4	35	81	-17	0.329	-12
25	113±1	2	252±5	44	77	-21	0.325	-16
30	121±1	10	261±1	52	77	-21	0.335	-6

Dog 4.

Time (mins)	$\overline{PR}$±SD (msec)	$\Delta\overline{PR}$ (msec)	$\overline{QT}$±SD (msec)	$\Delta\overline{QT}$ (msec)	HR (bts/min)	ΔHR	$\overline{QT}/\overline{RR}$	$\Delta\ \overline{QT}/\overline{RR}$
0	117±5	-	214±3	-	89	-	0.317	-
1	118±2	1	217±6	3	95	6	0.343	26
2	121±1	4	219±4	5	76	-13	0.277	-40
5	122±2	5	223±2	9	82	-7	0.305	-12
10	124±2	7	235±4	21	73	-16	0.286	-31
15	123±5	6	237±4	23	77	-12	0.304	-13
20	124±3	7	240±2	26	73	-16	0.292	-25
25	118±7	1	239±1	25	74	-15	0.295	-13
30	120±3	3	243±5	29	75	-14	0.304	-13

Table C.

The effects of sotalol, infused at a rate of 0.167 mg/kg/min, on HR, PR and QT intervals and QT/RR ratio in four conscious dogs pretreated with triiodothyronine. Each test was carried out in duplicate and the two test runs are shown for each dog. Recovery time of several days was allowed between tests. Where S.D. values are shown, these are calculated from a 6-beat sample of the ECG record at the times stated.

Dog 1.

Time (mins)	$\overline{PR}$ ± SD (sec)	Δ $\overline{PR}$ (msec)	$\overline{QT}$ ± SD (sec)	Δ $\overline{QT}$ (msec)	HR (bts/min)	Δ HR	$\overline{QT}/\overline{RR}$	Δ $\overline{QT}/\overline{RR}$
0	118 ± 7	-	220 ± 6	-	105	-	0.550	-
1	116 ± 5	-2	267 ± 4	47	87	-19	0.531	-19
2	117 ± 8	-1	271 ± 6	51	81	-25	0.532	-18
5	118 ± 7	-	292 ± 11	72	84	-22	0.618	68
10	119 ± 6	1	286 ± 5	66	102	-4	0.674	124
15	116 ± 4	-2	297 ± 6	77	109	3	0.679	129
20	127 ± 5	9	297 ± 5	77	104	-2	0.623	73
25	127 ± 6	9	340 ± 15	120	105	-1	0.668	118
30	119 ± 8	1	323 ± 3	103	103	-3	-	-
0	108 ± 10	-	221 ± 6	-	150	-	0.391	-
1	107 ± 12	-1	246 ± 7	25	129	-21	0.387	-4
3	106 ± 4	-2	257 ± 10	36	124	-26	0.400	9
5	107 ± 5	-1	251 ± 5	30	148	-2	0.407	16
10	114 ± 4	6	267 ± 11	46	151	1	0.488	97
15	111 ± 7	3	280 ± 9	39	157	7	0.540	149
20	115 ± 4	7	289 ± 16	68	131	-19	0.516	125
25	110 ± 3	2	272 ± 7	51	147	-3	0.596	205
30	112 ± 4	4	327 ± 7	104	147	-3	0.556	165

Dog 2.

Time (mins)	$\overline{PR}$ ± SD (msec)	Δ $\overline{PR}$ (msec)	$\overline{QT}$ ± SD (msec)	Δ $\overline{QT}$ (msec)	HR (bts/min)	Δ HR	$\overline{QT}/\overline{RR}$	Δ $\overline{QT}/\overline{RR}$
0	111 ± 2	-	258 ± 4	-	134	-	0.575	-
1	110 ± 6	-1	262 ± 5	4	134	-	0.586	01
2	114 ± 3	3	267 ± 5	9	129	-5	0.573	-2
5	111 ± 3	-	273 ± 4	15	131	-3	0.598	28
10	114 ± 3	3	289 ± 7	31	110	-24	0.532	-43
15	111 ± 2	-	289 ± 7	31	113	-21	0.547	-28
20	114 ± 3	3	316 ± 4	58	105	-29	0.550	-25
25	111 ± 2	-	297 ± 4	39	105	-29	0.521	-54
30	115 ± 4	4	330 ± 6	62	130	-4	0.692	117
0	108 ± 4	-	269 ± 5	-	125	-	0.545	-
1	111 ± 1	3	270 ± 3	1	144	19	0.646	101
2	112 ± 2	4	277 ± 8	8	141	16	0.651	106
5	106 ± 4	-2	282 ± 10	13	161	36	0.756	210
10	112 ± 4	4	313 ± 4	44	121	-3	0.250	-296
15	115 ± 4	7	332 ± 5	63	104	-21	0.578	33
20	111 ± 1	3	334 ± 4	65	107	-18	0.598	53
25	110 ± 2	2	334 ± 4	65	108	-17	0.602	57
30	109 ± 2	1	341 ± 1	72	100	-25	0.566	21

continued....

Table C continued.

Dog 3.

Time (mins)	$\overline{PR}$ ±SD (msec)	Δ $\overline{PR}$ (msec)	$\overline{QT}$ ±SD (msec)	Δ $\overline{QT}$ (msec)	HR (bt/min)	ΔHR	$\overline{QT}/\overline{RR}$	Δ $\overline{QT}/\overline{RR}$
0	112±2	-	227±3	-	130	-	0.492	-
1	113±2	1	231±4	4	125	-5	0.481	-11
2	112±2	-	235±5	8	121	-9	0.474	-18
5	110±1	-2	254±4	27	128	-2	0.542	50
10	114±2	2	269±1	42	122	-8	0.547	55
15	113±3	1	280±8	53	110	-20	0.513	21
20	111±3	-1	287±4	60	116	-14	0.555	63
25	113±1	1	295±5	68	97	-33	0.477	-15
30	113±1	1	299±5	72	96	-34	0.478	-14
0	111±1	-	221±2	-	149	-	0.549	-
1	112±1	1	231±2	10	135	-14	0.520	-29
2	116±3	5	236±5	15	128	-21	0.503	-46
5	115±3	4	245±4	24	132	-17	0.539	-10
10	117±2	6	257±5	36	118	-31	0.505	-44
15	116±3	5	272±2	51	118	-31	0.535	-14
20	115±2	4	286±7	65	104	-45	0.496	-53
25	116±2	5	294±3	73	103	-46	0.505	-44
30	115±3	4	299±2	78	100	-49	0.498	-51

Dog 4.

Time (mins)	$\overline{PR}$ ±SD (msec)	Δ$\overline{PR}$ (msec)	$\overline{QT}$ ±SD (msec)	Δ $\overline{QT}$ (msec)	HR (bt/min)	ΔHR	$\overline{QT}/\overline{RR}$	Δ $\overline{QT}/\overline{RR}$
0	113±5	-	241±5	-	128	-	0.514	-
1	114±3	1	247±3	6	128	-	0.527	13
2	114±4	1	266±5	25	123	-5	0.545	31
5	114±5	1	285±5	44	115	-13	0.546	32
10	117±3	4	301±2	60	111	-17	0.557	43
15	117±2	4	318±4	77	100	-28	0.530	16
0	113±3	-	240±6	-	156	-	0.624	-
1	118±6	5	250±2	10	116	-40	0.483	-141
2	116±3	3	253±3	13	136	-20	0.573	-51
5	116±3	3	278±5	38	134	-22	0.621	-3
10	118±6	5	315±9	75	119	-37	0.625	1
15	120±1	7	343±9	103	109	-47	0.623	-1
20	116±3	3	352±9	112	88	-68	0.657	33
25	118±2	5	349±9	109	105	-51	0.634	10
30	117±2	4	330±7	90	116	-40	0.638	14

Table D.

The effects of propranolol, infused at a rate of 0.067 mg/kg/min, on HR, PR and QT intervals and QT/RR ratio in four conscious dogs. Each test was carried out in duplicate and the two test runs are shown for each dog. Recovery time of several days was allowed between tests. Where S.D. values are shown, these are calculated from a 6-beat sample of the ECG record at each time stated.

Dog 1.

Time (mins)	$\overline{PR}$ ±SD (msec)	Δ PR (msec)	$\overline{QT}$ ±SD (msec)	ΔQT (msec)	HR (bts/min)	Δ HR	$\overline{QT}/\overline{RR}$	Δ QT/RR
0	109 ±3	-	217 ±4	-	89	-	0.326	-
1	110 ±1	1	220 ±5	3	84	-5	0.310	-16
2	118 ±5	9	220 ±2	3	67	-22	0.246	-80
5	124 ±4	15	228 ±3	11	75	-14	0.284	-42
10	128 ±4	19	227 ±4	10	86	-3	0.372	1
15	126 ±5	17	234 ±5	17	76	-13	0.296	-30
23	126 ±3	17	232 ±2	15	61	-28	0.235	-91
25	133 ±6	24	230 ±3	13	71	-18	0.274	-52
30	131 ±2	22	233 ±3	16	74	-15	0.287	-39
0	114 ±3	-	200 ±1	-	114	-	0.378	-
1	115 ±2	1	204 ±2	4	112	-2	0.379	1
2	123 ±4	9	206 ±4	6	106	-8	0.363	-15
5	122 ±5	8	208 ±6	8	99	-15	0.344	-34
10	140 ±6	26	210 ±2	10	108	-6	0.378	-
15	139 ±7	25	214 ±3	14	87	-27	0.312	-66
20	130 ±1	16	222 ±2	22	83	-31	0.308	-70
25	136 ±6	22	224 ±4	24	73	-41	0.274	-104
30	129 ±1	15	221 ±2	21	90	-24	0.330	-48

Dog 2.

Time (mins)	$\overline{PR}$ ±SD (msec)	Δ PR (msec)	$\overline{QT}$ ±SD (msec)	Δ QT (msec)	HR (bt/min)	Δ HR	$\overline{QT}/\overline{RR}$	Δ QT/RR
0	120±4	-	230±6	-	113	-	0.348	-
1	123±7	3	215±1	-15	101	-12	0.361	13
2	124±5	4	221±1	-9	75	-38	0.276	-72
5	129±4	9	210±3	-20	75	-38	0.261	-87
10	143±8	23	231±2	1	82	-31	0.318	-30
15	140±2	20	239±6	9	83	-30	0.333	-15
20	140±4	20	232±8	2	86	-27	0.329	-17
25	134±5	14	237±4	7	87	-26	0.330	-18
30	140±1	20	230±7	0	88	-25	0.336	-12
0	117±6	-	248±7	-	78	-	0.323	-
1	126±4	9	246±4	-2	76	-2	0.312	-11
2	127±9	10	256±4	8	64	-14	0.273	-50
5	135±6	18	264±4	16	60	-18	0.263	-60
10	141±8	24	266±6	18	59	-19	0.261	-62
15	135±5	18	264±7	16	61	-17	0.267	-56
20	135±5	18	264±8	16	68	-10	0.302	-21
25	140±6	23	264±3	16	72	-6	0.316	-7
30	141±2	24	266±8	18	65	-13	0.289	-34

continued....

Table D continued.

Dog 3.

Time (mins)	$\overline{PR}$ ±SD msec)	$\Delta\overline{PR}$ (msec)	$\overline{QT}$ ±SD (msec)	$\Delta\overline{QT}$ (msec)	HR (bt/min)	ΔHR	$\overline{QT}/\overline{RR}$	$\Delta\overline{QT}/\overline{RR}$
0	109±4	-	227±4	-	65	-	0.246	-
1	107±6	2	228±6	1	73	8	0.277	31
2	113±5	4	229±4	2	72	7	0.275	29
5	112±3	3	225±5	-2	67	2	0.251	5
10	112±5	3	230±2	3	67	2	0.257	11
15	117±6	8	228±2	1	80	15	0.304	58
20	122±5	13	225±3	-2	77	12	0.321	75
25	119±8	10	220±2	-7	78	13	0.286	40
30	122±6	13	224±4	-3	81	16	0.302	56
0	110±6	-	219±6	-	94	-	0.343	-
1	115±2	5	222±6	3	86	-8	0.318	-25
2	113±2	3	218±8	-1	104	10	0.378	35
5	126±7	16	225±6	6	63	-31	0.236	-107
10	131±5	21	224±3	5	69	-25	0.258	-85
15	133±3	23	220±9	1	80	-14	0.293	-50
20	136±9	26	215±6	-4	90	-4	0.322	-21
25	131±4	21	215±4	-4	82	-12	0.294	-49
30	132±4	22	222±2	3	76	-18	0.281	-62

Dog 4.

Time (mins)	$\overline{PR}$ ±SD (msec)	$\Delta\overline{PR}$ (msec)	$\overline{QT}$ ±SD (msec)	$\Delta\overline{QT}$ (msec)	HR (bt/min)	ΔHR	$\overline{QT}/\overline{RR}$	$\Delta\overline{QT}/\overline{RR}$
0	107±4	-	197±4	-	106	-	0.348	-
1	114±5	7	195±1	-2	110	4	0.357	9
2	114±4	7	200±3	3	110	4	0.367	19
5	114±5	7	201±1	4	104	-2	0.348	-
10	119±2	12	202±3	5	102	-4	0.343	-5
15	124±4	17	198±4	1	107	1	0.353	5
20	121±6	14	204±4	7	96	-10	0.326	-22
25	121±1	14	207±2	10	101	-5	0.348	-
30	126±2	19	205±4	8	98	-8	0.335	-13
0	111±3	-	212±4	-	92	-	0.325	-
1	111±1	-	215±4	3	96	4	0.344	19
2	116±3	5	214±4	2	91	-1	0.325	-
5	121±1	10	217±4	5	78	-14	0.282	-43
10	123±3	12	217±3	5	89	-3	0.322	-3
15	129±4	18	225±2	13	77	-15	0.389	-36
20	127±2	16	223±4	11	82	-10	0.305	-20
25	131±2	20	226±3	14	83	-9	0.313	-12
30	133±5	22	225±5	13	82	-10	0.308	-17

Table E.

The effects of propranolol, infused at a rate of 0.067 mg/kg/min, on HR, PR and QT intervals and QT/RR ratio in four conscious dogs pretreated with carbimazole. Where S.D. values are shown, these are calculated from a 6-beat sample of the ECG record at each time stated.

Dog 1.

Time (mins)	$\overline{PR} \pm SD$ (msec)	$\Delta \overline{PR}$ (msec)	$\overline{QT} \pm SD$ (msec)	$\Delta \overline{QT}$ (msec)	HR (bts/min)	Δ HR	$\overline{QT}/\overline{RR}$	$\Delta \overline{QT}/\overline{RR}$
0	125±5	-	219±7	-	82	-	0.289	-
1	114±8	-11	216±9	-3	54	-28	0.196	-93
2	124±6	-1	231±2	12	55	-27	0.213	-76
5	127±6	2	223±5	4	66	-16	0.245	-44
10	130±3	5	220±4	1	73	-9	0.268	-21
15	128±4	3	221±4	2	84	2	0.310	21
20	134±6	8	224±4	5	66	-16	0.248	-41
25	128±5	3	214±5	-5	92	10	0.327	38
30	139±4	14	218±6	-1	61	-21	0.223	-66

Dog 2.

Time (mins)	$\overline{PR} \pm SD$ (msec)	$\Delta \overline{PR}$ (msec)	$\overline{QT} \pm SD$ (msec)	$\Delta \overline{QT}$ (msec)	HR (bts/min)	Δ HR	$\overline{QT}/\overline{RR}$	$\Delta \overline{QT}/\overline{RR}$
0	117±2	-	221±2	-	84	-	0.329	-
1	122±2	5	225±4	4	78	-6	0.292	-37
2	126±4	9	233±3	12	66	-18	0.257	-72
5	126±4	9	227±5	6	118	34	0.445	116
10	132±2	15	234±7	13	63	-21	0.245	-84
15	135±3	18	234±3	13	71	-13	0.276	-53
20	139±4	22	241±2	20	61	-23	0.245	-84
25	136±3	19	240±4	19	71	-13	0.284	-45
30	140±4	23	242±5	21	68	-16	0.274	-55

continued....

Table E continued.

Dog 3.

Time (sec)	$\overline{PR}$±SD (msec)	Δ $\overline{PR}$ (msec)	$\overline{QT}$ ±SD (msec)	Δ$\overline{QT}$ (msec)	HR (bt/min)	ΔHR	$\overline{QT}/\overline{RR}$	Δ$\overline{QT}/\overline{RR}$
0	112±2	-	210±5	-	94	-	0.329	-
1	114±2	2	216±3	6	80	-14	0.288	-41
2	117±3	5	217±4	7	75	-19	0.271	-58
5	120±2	8	219±7	9	86	-8	0.314	-15
10	126±3	14	221±2	11	78	-16	0.287	-42
15	128±2	16	223±3	13	89	-5	0.331	2
20	131±1	19	225±2	15	83	-11	0.311	-18
25	131±3	19	224±3	14	79	-15	0.295	-34
30	127±3	15	226±3	16	82	-12	0.309	-20

Dog 4.

Time (mins)	$\overline{PR}$±SD (msec)	Δ$\overline{PR}$ (msec)	$\overline{QT}$±SD (msec)	Δ$\overline{QT}$ (msec)	HR (bt/min)	ΔHR	$\overline{QT}/\overline{RR}$	Δ$\overline{QT}/\overline{RR}$
0	112±3	-	228±3	-	100	-	0.380	-
1	113±2	1	225±5	-3	109	9	0.409	29
2	117±3	5	220±3	-8	93	-7	0.341	-39
5	118±3	6	230±3	2	79	-21	0.303	-77
10	117±4	5	234±2	6	79	-21	0.308	-72
15	123±2	11	236±5	8	81	-19	0.319	-61
20	124±1	12	231±1	3	80	-20	0.308	-72
25	123±5	11	236±4	8	79	-21	0.311	-69
30	128±4	16	223±3	-5	75	-25	1.279	-101

Table F.

The effects of propranolol, infused at a rate of 0.067 mg/kg/min, on HR, PR and QT intervals and QT/RR ratio in four conscious dogs pretreated with triiodothyronine. Recovery time of several days was allowed between tests. Where S.D. values are shown, these are calculated from a 6-beat sample of the ECG record at each time stated.

Dog 1.

Time (mins)	$\overline{PR}$ ±SD (msec)	Δ $\overline{PR}$ (msec)	$\overline{QT}$ ±SD (msec)	Δ $\overline{QT}$ (msec)	HR (bt/min)	ΔHR	$\overline{QT}/\overline{RR}$	Δ$\overline{QT}/\overline{RR}$
0	105±1	-	227±4	-	141	-	0.533	-
1	109±3	4	227±3		119	-22	0.450	-83
2	111±2	6	231±2	4	143	2	0.551	18
5	113±1	8	240±1	13	121	-20	0.484	-49
10	114±3	9	240±7	13	125	-16	0.500	-33
15	115±3	10	244±3	17	122	-19	0.496	-37
20	114±3	9	244±5	17	119	-22	0.484	-49
25	120±2	15	245±3	18	117	-24	0.478	-55
30	127±5	15	238±3	11	113	-28	0.448	-85
0	114±3	-	239±1	-	145	-	0.581	-
1	116±2	2	248±2	9	144	-1	0.595	14
2	118±3	4	251±2	12	135	-10	0.565	-16
5	118±2	4	246±3	7	147	2	0.603	22
10	117±5	3	262±2	23	128	-17	0.559	-22
15	118±4	4	254±5	15	130	-15	0.550	-31
20	126±3	12	252±1	13	141	-4	0.592	11
25	125±3	11	262±2	23	124	-21	0.541	-40
30	124±3	10	250±2	11	136	-9	0.567	-14

Dog 2.

Time (mins)	$\overline{PR}$ ±SD (msec)	Δ $\overline{PR}$ (msec)	$\overline{QT}$ ±SD (msec)	Δ $\overline{QT}$ (msec)	HR (bt/min)	ΔHR	$\overline{QT}/\overline{RR}$	Δ $\overline{QT}/\overline{RR}$
0	97±3	-	203±3	-	172	-	0.582	-
1	102±3	5	211±2	8	151	-21	0.531	-51
2	102±2	5	224±6	21	141	-31	0.526	-56
5	111±1	14	235±4	32	129	-43	0.505	-77
10	112±2	15	235±4	32	130	-42	0.509	-73
15	111±3	14	237±4	34	128	-44	0.506	-76
20	114±1	17	237±2	34	134	-38	0.529	-53
25	110±1	13	236±4	33	134	-38	0.527	-55
30	121±1	24	235±5	32	129	-43	0.505	-77
0	110±2	-	231±4	-	142	-	0.547	-
1	111±2	1	234±5	3	148	6	0.577	30
2	115±2	4	239±1	8	132	-10	0.526	-21
5	116±2	5	242±3	11	134	-8	0.540	-7
10	118±4	7	236±3	5	135	-7	0.531	-16
15	120±1	10	237±3	6	150	8	0.592	45
20	120±1	10	235±4	4	141	-1	0.552	5
25	121±3	11	237±2	6	140	-2	0.553	6
30	121±6	11	238±2	7	141	-1	0.559	12

continued....

Table F continued.

Dog 3.

Time (mins)	$\overline{PR}$ ±SD (msec)	Δ PR (msec)	$\overline{QT}$ ±SD (msec)	ΔQT (msec)	HR (bts/min)	ΔHR	$\overline{QT}/\overline{RR}$	Δ QT/RR
0	116 ± 9	-	213 ± 6	-	107	-	0.390	-
1	116 ± 6	-	238 ± 6	25	98	-9	0.390	-
2	131 ± 8	15	236 ± 5	23	96	-11	0.377	-13
5	118 ± 4	2	241 ± 3	28	104	-3	0.416	26
10	117 ± 2	1	234 ± 4	21	104	-3	0.405	15
15	121 ± 6	5	234 ± 7	21	99	-8	0.388	-2
20	123 ± 4	7	227 ± 7	14	107	-	0.404	14
25	119 ± 5	3	239 ± 5	26	93	-14	0.369	-21
30	123 ± 5	7	222 ± 4	9	96	-11	0.355	-35
0	105 ± 4	-	229 ± 5	-	140	-	0.535	-
1	107 ± 4	2	230 ± 6	1	156	15	0.596	61
2	116 ± 4	11	232 ± 6	3	124	-16	0.480	-55
5	117 ± 4	12	230 ± 4	1	147	7	0.564	29
10	114 ± 6	9	223 ± 4	-6	170	30	0.633	98
15	114 ± 4	9	230 ± 6	1	148	8	0.567	32
20	114 ± 6	9	222 ± 2	-7	143	3	0.530	-5
25	116 ± 4	11	226 ± 5	-3	160	20	0.604	69
30	117 ± 3	12	229 ± 4	-	157	17	0.601	66

Dog 4.

Time (mins)	$\overline{PR}$ ± SD (msec)	Δ $\overline{PR}$ (msec)	$\overline{QT}$ ± SD (msec)	Δ $\overline{QT}$ (msec)	HR (bts/min)	Δ HR	$\overline{QT}/\overline{RR}$	Δ $\overline{QT}/\overline{RR}$
0	106 ± 4	-	229 ± 4	-	126	-	0.478	-
1	107 ± 2	1	221 ± 2	-8	146	20	0.537	59
2	102 ± 6	-4	216 ± 4	-13	150	24	0.541	63
5	111 ± 2	5	223 ± 7	-6	124	-2	0.460	-18
10	114 ± 2	8	227 ± 4	-2	131	5	0.496	18
15	119 ± 4	13	245 ± 7	16	112	-14	0.456	-22
20	120 ± 3	14	249 ± 9	20	110	-16	0.457	-21
25	124 ± 4	18	259 ± 8	30	111	-15	0.477	-1
30	124 ± 4	18	261 ± 1	32	103	-23	0.450	-28
0	110 ± 3	-	282 ± 9	-	111	-	0.524	-
1	106 ± 4	-4	252 ± 6	-30	145	34	0.540	14
2	107 ± 3	-3	264 ± 12	-18	156	45	0.684	160
5	113 ± 3	3	219 ± 10	-63	119	8	0.434	-90
10	111 ± 3	1	225 ± 12	-57	145	34	0.544	20
15	118 ± 6	7	251 ± 21	-31	132	21	0.553	29
20	119 ± 4	9	271 ± 9	-11	124	13	0.560	36
25	113 ± 11	3	268 ± 11	-14	114	3	0.507	-17
30	122 ± 14	11	252 ± 14	-30	125	14	0.523	-1

Table G.

The effects of nadolol, infused at a rate of 0.167 mg/kg/min, on HR, PR and QT intervals and QT/RR ratio in two conscious dogs. Where S.D. values are shown, these are calculated from a 6-beat sample of the ECG record at each time stated.

	Time (mins)	PR ± SD (msec)	Δ PR (msec)	QT ± SD (msec)	Δ QT (msec)	HR (bts/min)	Δ HR	QT/RR	ΔQT/RR
Dog 1.	0	112±2	-	210±2	-	104	-	0.364	-
	1	119±4	7	211±2	1	89	-15	0.313	-51
	2	117±5	5	213±4	3	91	-13	0.323	-41
	5	117±5	5	223±4	13	97	-7	0.360	-4
	10	118±5	6	219±2	19	93	-11	0.339	-25
	15	116±3	4	219±3	19	94	-10	0.343	-21
	20	115±5	3	217±2	17	94	-10	0.340	-24
Dog 2.	0	117±3	-	237±2	-	63	-	0.249	-
	1	119±1	8	244±4	7	70	7	0.285	36
	2	120±2	9	254±4	17	61	-2	0.258	9
	5	120±5	9	253±2	16	58	-5	0.245	-4
	10	112±4	1	246±2	9	69	6	0.283	34
	15	110±2	-1	262±5	25	51	-12	0.223	-25
	20	114±3	3	249±2	12	66	3	0.274	25

Table H.

The effects of nadolol, infused at a rate of 0.167 mg/kg/min, on HR, PR and QT intervals and QT/RR ratio in two conscious dogs pretreated with carbimazole. Where S.D. values are shown, these are calculated from a 6-beat sample of the ECG record at the time stated.

	Time (mins)	PR ± SD (msec)	Δ PR (msec)	QT ± SD (msec)	Δ QT (msec)	HR (bts/min)	ΔHR	QT/RR	ΔQT/RR
Dog 1.	0	114±2	-	205±4	-	98	-	0.336	-
	1	112±4	-2	209±1	3	95	-3	0.331	-5
	2	116±5	2	210±1	4	93	-5	0.325	-10
	5	115±3	1	213±3	-3	86	-12	0.305	-31
	10	114±3	-	208±2	2	98	-	0.340	4
	15	115±3	1	209±4	3	101	3	0.352	16
	20	110±3	4	204±3	-2	91	-7	0.309	-27
	2	114±2	-	206±3	-	105	8	0.364	28
Dog 2.	0	114±4	-	214±4	-	88	-	0.314	-
	1	114±3	-	217±6	3	88	-	0.317	4
	2	118±5	4	228±5	14	84	-4	0.329	5
	5	114±3	-	231±1	17	70	-1	0.270	-44
	10	113±3	-1	231±2	17	64	-4	0.323	7
	15	117±4	3	234±3	20	79	-9	0.303	-6
	20	113±5	-1	227±3	13	82	-6	0.310	-4
	25	112±1	-2	230±1	16	82	-6	0.314	-
	30	113±2	-1	228±4	14	84	-4	0.317	5

Table I.

The effects of nadolol, infused at a rate of 0.167 mg/kg/min, on HR, PR and QT intervals and QT/RR ratio in two conscious dogs pretreated with triiodothyronine. Where S.D. values are shown, these are calculated from a 6-beat sample of the ECG record at the time stated.

	Time (mins)	PR ± SD (msec)	Δ PR (msec)	QT ± SD (msec)	Δ QT (msec)	HR (bts/min)	Δ HR	QT/RR	ΔQT/RR
Dog 1.	0	108±4	-	233±1	-	129	-	0.501	-
	1	112±2	4	232±5	-1	148	19	0.572	71
	2	114±2	6	244±2	11	147	18	0.598	97
	5	112±1	4	237±4	4	147	18	0.581	80
	10	111±2	3	227±8	-6	143	14	0.541	40
	15	112±3	4	238±4	5	139	10	0.551	50
	20	111±8	3	259±3	26	88	-41	0.380	-121
	25	110±4	2	247±3	14	108	-21	0.445	-56
	30	112±1	1	248±3	15	94	-35	0.388	-113
Dog 2.	0	110±2	-	242±5	-	157	-	0.635	-
	1	112±2	2	239±2	-3	143	-14	0.570	-63
	2	118±4	9	257±2	15	129	-28	0.552	-81
	5	115±1	5	266±3	24	131	-26	0.581	-52
	10	116±6	8	266±6	24	138	-19	0.612	-21
	15	113±3	8	270±2	28	124	-33	0.558	-75
	20	118±3	8	266±3	24	125	-32	0.554	-79
	25	116±3	6	267±2	25	130	-27	0.578	-55
	30	110±5	-	266±3	24	125	-32	0.554	-79

Table J.

EMR and K_a values for isolated guinea-pig atria.

Parameter	CONTROL		6-OHDA -	
	Chronotropic	Inotropic	Chronotropic	Inotropic
EMR				
NA	1.07 (40)	3.26 (45)	1.06 (35)	3.18 (38)
Salb	2.83 (36)	32.4 (42)	0 (44)	0 (32)
K_D Practolol				
Isop	1.74×10^{6} (15)	1.61×10^{6} (16)	3.46×10^{6} (16)	3.23×10^{6} (12)
NA	2.28×10^{7} (12)	2.36×10^{7} (15)	1.71×10^{7} (22)	1.85×10^{7} (15)
Salb	6.30×10^{4} (14)	6.21×10^{4} (13)	-- (10)	-- (14)
K_D Ro-03 7894				
Isop	6.15×10^{5} (12)	6.27×10^{5} (10)	6.40×10^{5} (15)	6.11×10^{5} (11)
NA	1.38×10^{5} (11)	3.95×10^{5} (11)	1.45×10^{5} (14)	3.80×10^{5} (10)
Salb	1.16×10^{4} (10)	4.23×10^{4} (10)	-- (12)	-- (11)
K_D ICI 118 551				
Isop	4.39×10^{6} (15)	5.40×10^{6} (14)	4.25×10^{6} (14)	5.07×10^{6} (12)
NA	3.85×10^{6} (14)	3.55×10^{6} (13)	3.77×10^{6} (11)	3.62×10^{6} (18)
Salb	7.80×10^{6} (14)	8.40×10^{6} (15)	-- (12)	-- (14)
K_D Salbutamol				
Isop	--	--	2.61×10^{5} (18)	2.46×10^{5} (17)
NA	--	--	1.50×10^{6} (17)	1.38×10^{6} (14)

Equipotent Molar Ratios (EMR) are calculated with respect to Isoprenaline (1)

Figures in brackets indicate number of estimates.

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